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Defining the factors that affect solute permeation of gap junction channels☆

Virginijus Valiunas *, Ira S. Cohen, Peter R. Brink *

Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY 11794, USA

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ABSTRACT

This review focuses on the biophysical properties and structure of the pore and vestibule of homotypic gap junction channels as they relate to channel permeability and selectivity. Gap junction channels are unique in their sole role to connect the cytoplasm of two adjacent cells. In general, these channels are considered to be poorly selective, possess open probabilities approximating unity, and exhibit mean open times ranging from milliseconds to seconds. These properties suggest that such channels can function as delivery pathways from cell to cell for solutes that are significantly larger than monovalent ions. We have taken quantitative data from published works concerning unitary conductance, ion flux, and permeability for homotypic connexin 43 (Cx43), Cx40, Cx26, Cx50, and Cx37, and performed a comparative analysis of conductance and/or ion/solute flux versus diffusion coefficient. The analysis of monovalent cation flux portrays the pore as equivalent to an aqueous space where hydrogen bonding and weak interactions with binding sites dominate. For larger solutes, size, shape and charge are also significant components in determining the permeation rate. This article is part of a Special Issue entitled: Gap Junction Proteins edited by Jean Claude Herve.

Contents

1. Introduction

Presently, there are at least 21 known human connexin genes [1]. For each connexin protein there is a documented homotypic gap junction channel form composed of 12 identical connexins where 6 identical connexins form a hemichannel in each cell of a cell pair. Heterotypic gap junction channels are formed by two non-identical hemichannels. Heteromeric channels are formed by hemichannels that are composed of more than one connexin type. For any two mutually compatible

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* Corresponding authors at: Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY 11794-8661, USA.
E-mail addresses: virginijus.valiunas@stonybrook.edu (V. Valiunas), peter.brink@stonybrook.edu (P.R. Brink).

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connexins there are, in theory, 196 gap junction channel types possible [2], either as a heteromeric or heterotypic form. In principle, it is therefore possible to have hundreds if not thousands of different channel types. The in vivo state of affairs for connexin expression and thus functionality is clearly complicated by the potential for multiple connexin expression [1,3,4], which is hampered by yet another factor, the rapid turnover rates of the connexin protein subunits [5].

To understand the biophysical properties of gap junction channels composed of connexins, researchers have used connexin deficient cell types transfected with DNA for a single connexin. This allows analysis of the physical properties that govern homotypic gap junction channel function. A number of groups have studied properties such as fluorescent probe and second messenger permeability as well as unitary conductance to better understand the molecular nature of gap junction permeability and selectivity [6,7]. In this review we will use data from a number of published works to elucidate some of the experimentally deduced properties of gap junction pore selectivity/permeability and discuss the possible underlying molecular mechanisms.

1.1. The anatomy of the pore

Each connexin is composed of 4 alpha-helices which are membrane spanning domains, 2 extracellular loops, one cytoplasmic loop, an N-terminus and a C-terminus [6,8–10]. Crystal structures of two homotypic gap junction channels composed of Cx26 or Cx43 have been determined to a resolution of 0.3–0.4 nm for Cx26 and 0.75 nm in the membrane plane and 2.1 nm in the vertical plane for Cx43. Both structures reveal a pore with a minimum of approximately 1.4 nm [11,12], a width bigger than the hydrated diameter of most permeant ions. To help define which domains of a connexin contribute to the lining of the pore, the substitution cysteine accessibility method (SCAM) has been employed extensively with Cx46 and to a lesser degree on Cx32 and Cx26 [13]. The combined studies strongly suggest that part of the N-terminus, the first transmembrane domain (TM1), and portions of the extracellular loops are significant contributors to the lining of the pore [8].

1.2. Channel properties apart from the pore itself that influence permeability

Two important measurements of all channels are their mean open and closed times. Mean open time (along with single channel conductance) determines the number of ions that will transit a channel from one compartment to another within a cell, from intracellular to extracellular space, or in the case of gap junction channels intercellularly. The mean open time of many channels is strongly influenced by voltage. Gap junction channels, also display voltage dependent behavior but atypically spend most of their time in the open state. For homotypic channels, voltage dependence is symmetrical such that when transjunctional voltage is 0 the channel remains open more often than closed. A number of studies have demonstrated voltage dependence and determined that mean open time declines with increased transjunctional voltage [14–17]. Since at 0 transjunctional voltage gap junctions are open more than 50% of the time, it is not surprising that Cx43 homotypic channels have a mean open time that exceeds their mean closed time, averaging 2.5 s in the open state versus 0.7 s in the closed state [18]. These values are 2–3 orders of magnitude longer in duration than K^+, Na^+, and Ca^{2+} channels. Such long open times of Cx43 might permit solutes of greater mass as large as 4–5 kD to traverse this gap junction channel, particularly, if they are long with a narrow radius implying a large aspect ratio [19].

Another potentially significant factor regarding permeability and selectivity is access to the pore. Depending on the sign of net fixed charge near the pore orifice, it can effectively repel or attract charged solutes creating the basis for charge selectivity [20].

2. A broad spectrum of permeant ions and solutes

Initial observations of single gap junction channels revealed unitary conductances of over one hundred picosiemens [21,22]. Subsequent identification of the connexin family [23] resulted in numerous studies where measurements of homotypic channels revealed that connexins were permeable to more than one monovalent ion and also allowed the transit of fluorescent probes [6,14,24–26], polypeptides [27], and oligonucleotides [19].

Initially, the observation that varied ions and solutes are able to transit from one cell to another via gap junctions resulted in the belief that the channels were non-selective, with the solute size and charge as the only rate limiting steps [6]. However, a number of studies, including that of Elfgang et al. [28], showed that specific homotypic and heterotypic gap junction channels displayed different apparent permeabilities to a number of fluorescent probes, leading to the suggestion that not all connexins function the same, especially for solutes in the size range of second messengers [26]. Data like that shown by Elfgang et al. and others [26,29,30] have led many to speculate: is there any evidence for cation or anion selectivity in gap junction channels?

2.1. Monovalent ions: are gap junction channels selective or simply non-discriminant conduits?

In the early 1960s when the search for ion selective microelectrodes began Eisenman predicted selectivity sequences based on binding to ion-selective glasses of different compositions [31]. He concluded that for the 5 alkali metal cations only 11 out of a possible 120 sequences are relevant to chemistry and biology [31]. Gap junction channels appear to possess conductive properties for monovalent cations most like Eisenman sequences I (Cs > Rb > K > Na > Li) or II (Rb > Cs > K > Na > Li) [31]. Table 1 gives the sequences for a number of connexins for which there is sufficient data on unitary conductances and where Cx43 and Cx40 are consistent with Eisenman sequences I and II. Both sequences represent a circumstance where solute binding to a site is weak based on electrostatic attraction and energy of hydration [32]. Cx37 and Cx50 are two examples that do not quite fit the simple conduit model for gap junction pores. Cx50 is an Eisenman I sequence but the slope of the regression coefficient is nearly twice that of Cx43 and Cx40, indicating that some additional solute-solvent or solute pore interactions might be occurring. Cx26 is consistent with an Eisenman I or II sequence but with only two data points it is not possible to assign a precise sequence. In the case of Cx37, the slope of the regression coefficient is intermediate to Cx50 and the others. Further, the best or closest Eisenman sequence for Cx37 is a series IV sequence yet another indicator that solutes are interacting with the pore walls and/ or solvents to a greater extent than, for example, in Cx43.

Another way to view the single channel conductance data is to compare the unitary conductance for each homotypic channel to a measure of the same ion's mobility in free solution. Fig. 1A is a plot of unitary conductances (normalized to Cs^+) for Cx43, Cx40, Cx50, Cx37, and Cx26 homotypic channels on the ordinate versus their respective diffusion coefficients on the abscissa. If free diffusion was occurring with no selectivity, the fractional change in diffusion coefficient should match the decline in single channel conductance with change in permeant ion. This is true except Cx50. Fits of the data in Fig. 1A to a linear regression produced the following slopes: $0.5139 \pm 0.06552/cm^2s^{-1} \times 10^{-5}$ (Cx43), $0.5544 \pm 0.02908/cm^2s^{-1} \times 10^{-5}$ (Cx40), $0.724 \pm 0.1496\ /cm^2s^{-1} \times 10^{-5}$ (Cx37), $1.066 \pm 0.2853/cm^2s^{-1} \times 10^{-5}$ (Cx50), and $0.3956 \pm$

Table 1
Connexin permselectivity sequences for monovalent cations.

Cx43 $Rb^+ \geq Cs^+ > K^+ > Na^+ > TMA^+ > Li^+ > TEA^+$	[24]
Cx40 $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+ > TMA^+ > TEA^+$	[25]
Cx26 $K^+ > Na^+$	[33]
Cx37 $K^+ > Cs^+ > Rb^+ > Na^+ > Li^+ > TMA^+$	[34]
Cx50 $Cs^+ > K^+ > Na^+$	[35,36]

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