UltraRapid Communication

Modifications in the Biophysical Properties of Connexin43 Channels by a Peptide of the Cytoplasmic Loop Region

Akiko Seki, Heather S. Duffy, Wanda Coombs, David C. Spray, Steven M. Taffet, Mario Delmar

Abstract—Connexin43 (Cx43) channels reside in at least 3 states: closed, open, or residual. It is hypothesized that the residual state results from the interaction of an intracellular “gating element” with structures at the vestibule of the pore. Recently, we showed in vitro that there is an intramolecular interaction of the carboxyl-terminal domain (referred to as “CT”) with a region in the cytoplasmic loop of Cx43 (amino acids 119 to 144; referred to as “L2”). Here, we assessed whether the L2 region was able to interact with the gating particle in a functional channel. Cx43 channels were recorded in the presence of a peptide corresponding to the L2 region, delivered via the patch pipette. This manipulation did not modify unitary conductance, but decreased the frequency of transitions into the residual state, prolonged open time, and altered the voltage dependence of the channel in a manner analogous to that observed after truncation of the CT domain. The latter correlated with the ability of the peptide to bind to the CT domain, as determined by mirror resonance spectroscopy. Overall, we propose that the L2 acts as a “receptor” that interacts with a flexible intracellular gating element during channel gating. The full text of this article is available online at http://circres.ahajournals.org. (Circ Res. 2004;95:e22-e28.)

Key Words: connexin43 | gap junction | pH gating

Gap junction channels are formed by oligomerization of connexins. The most abundant connexin in heart is connexin43 (Cx43). Electrophysiological analysis shows that Cx43 channels can reside in at least 3 distinct states: closed, open, and residual.1,2 Biophysical studies suggest that open-to-residual transitions (“Fast Vj gating”) occur when a gating element moves into the cytoplasmic vestibule of the channel, causing a narrowing of the pore, an increase in charge density at the vestibule of the channel, and a consequent change in perm-selectivity.3 Whereas previous studies have tried to identify the structural domain corresponding to the gating element,3–7 the location of the channel vestibule (which would act as a “receptor” for the gating particle)5 has been more elusive.

Recently, we determined the structural characteristics of a peptide corresponding to the second half of the cytoplasmic loop of Cx43 (amino acids 119 to 144; referred to as “L2”)8 and demonstrated its ability to bind to the carboxyl terminal (CT) domain of the same protein. Hence, we proposed that the L2 region acted as the receptor for the gating particle. The latter has awaited confirmation in a system in which functional channels can be assessed.

Small peptides are commonly used to competitively interfere with protein–protein interactions.9 Here, we reason that if the L2 region acts as a receptor for the gating particle, channel properties (such as residence in the residual state) could be impaired by a peptide of the L2 region that, by binding to the gating element, could competitively inhibit its association to the endogenous loop domain. Here, we tested the effect of a peptide corresponding to the L2 region on the electrophysiological properties of Cx43 channels. The results show that this peptide reduced the frequency of channel transitions into the residual state, prolonged open time and transition rate, and modified the voltage dependence of the junctional current. These modifications were analogous to those previously observed when the CT domain was deleted from the Cx43 sequence.5 Our data indicate that the L2 region interacts (directly or indirectly) with a separate region of Cx43 (likely the CT domain) during channel gating. Our data further support the notion that the L2 region is in close structural association with the pore and may be part of the channel vestibule.10,11 Moreover, we show that small peptides can be used to interfere with gap junction function, a strategy that may be useful for rational design of gap junction pharmacophores.

Materials and Methods

Cell Culture and Transfection of Neuroblastoma Cells

Experiments were conducted in communication-deficient murine neuroblastoma cells (N2A). The cells were maintained in DMEM (Life Technologies) (supplemented, as described by Moreno et al8).

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Transient transfections (1 µg of plasmid DNA; Effectene; Qiagen, Valencia, Calif) were performed in N2A cells grown to 20% to 30% confluence. Experimental recordings were obtained 48 to 58 hours after transfection. For these experiments, rat cardiac Cx43 cDNA was subcloned into the bicistronic expression vector pREStable enhanced green fluorescence protein (eGFP) (Clontech, Palo Alto, Calif), enabling the coexpression of the connexin protein and, as a separate product, the eGFP. For the studies of voltage dependence, an inducible Cx43 expression system was used (see Zhong et al). This system (LacSwitch II; Stratagene) uses a stably transfected lac-repressor and a lac-sensitive promoter to control the expression of the gene of interest. N2A cells transfected with both the repressor and POPRS1-Cx43 were kindly provided by Dr. Alonso Moreno (Indiana University School of Medicine). These cells were maintained in DMEM and supplemented, as described by Zhong et al, with 1 mg/mL geneticin (G418 sulfate) and 1 µg/mL hygromycin. Isopropyl-β-D-thiogalactopyranoside (0.1 to 2 mmol/L) was added for 10 to 12 hours for induction of Cx43.

**Solutions**

The composition of the internal and external solutions was the same as described by Moreno et al. When necessary, coupling was decreased by superfusion with octanol (0.5 to 2 mmol/L). The L2 peptide (amino acid sequence 119 to 144; see Duffy et al) or an L2 peptide holding the mutation H126K-I130N was added to the pipette solution to a final concentration of 0.1 mmol/L. Chemical compounds for cell culture and for the preparation of the stock solutions were purchased from Sigma, St. Louis, Mo.

**Electrophysiology**

Transiently transfected N2A cells were illuminated with monochromatic light (495 nm) and emitted fluorescence passed through a bandpass (520 to 560 nm) filter to monitor eGFP expression. Those cell pairs identified positive for eGFP were targeted for electrophysiological analysis. Recordings were obtained using conventional dual-patch clamp techniques. Single channel recordings were obtained at a transjunctional voltage (Vj) of +60 mV. Input resistance was continuously monitored to ensure proper voltage control at the junction. Cell pairs showing chord membrane conductances >400 pS (pico siemens) (measured between −40 mV and +20 mV) were discarded. All signals were acquired in broad band, digitized, and stored for offline analysis. Analysis of data were performed using the pClamp suite of programs (Versions 8.2; Axon Instruments, Union City, Calif). Currents were filtered offline (0.1 to 1 kHz) and digitally sampled at 2 to 10 kHz. Most determinations of unitary conductance were obtained in the presence of 0.5 to 2 mmol/L octanol. In some cases, however, recordings were obtained from cells for which only 1 or 2 functional channels were spontaneously detected. Histograms of events (see also below) were obtained from channels recorded during repetitive 10- to 20-second steps to Vj=-60 mV. Channel events were manually selected and measured. Only events lasting 20 ms or more (to reduce filtering artifacts) were included in the event histogram. Unitary currents were divided by the driving force (+60 mV) to obtain unitary conductance. All-points histograms of digitized current traces, as well as frequency (percentage of total events) distribution histograms, were constructed using Origin (version 7.0; Microcal, Northhampton, Mass). Rate of transition between states was measured in 100 transitions acquired between 30 and 60 minutes after patch break. The measured transitions were chosen for their high signal-to-noise ratio. For these measurements, transitions were filtered at 1 kHz. The frequency of transitions into the residual state was determined from the first 100 transitions recorded 30 minutes after patch break. This standard was arbitrarily chosen to avoid possible variations in the residence of a given state that may have occurred as a result of time after patch break.

**Peptide Synthesis**

Peptidic fragments of the cytoplasmic loop region of Cx43 (L2 peptide and peptide H126K-I130N; see text below) were synthesized (>95% purity) at the Laboratory for Macromolecular Analysis at the Albert Einstein College of Medicine using Fmoc (9-fluorenylmethoxycarbonyl) chemistry on an Applied Biosystems 430A automated peptide synthesizer.

**Mirror Resonance Spectroscopy**

Mirror resonance spectroscopy (MRS) experiments were performed using an IAsys apparatus as described previously. Recombinant Cx43CT was used as ligand. Concentration dependence curves were best-fit by a single exponential function, as predicted by first-order rate kinetics.

**Results**

A Peptide of the L2 Region Interfered with the Residual State

The unitary conductance, residence states, open time, rate of transition, and voltage dependence of Cx43 channels and of Cx43 channels lacking the carboxyl terminal domain have been described previously. Here, we asked whether these Cx43 properties were affected by the presence of the L2 peptide. Figure 1 shows recordings of single gap junction channel activity obtained from an N2A cell pair transiently transfected with Cx43. The L2 peptide was diluted in the internal pipette solution in both cells of the pair. An all-points histogram is presented at the right of the trace. Figure 1A shows a recording obtained when only a single channel was active. Clearly, the channel transited between only the closed state and a single conductive (open) state; thus, although the unitary conductance (114.8 pS) was similar to that previously reported for Cx43 channels, in this case, no residual state was observed. Similarly, the trace in Figure 1B shows 2 conductive levels (O1 and O2) and no residual state. Unitary conductance: 112 pS.

Figure 1. A peptide of the L2 region abolished the residual state. A and B, Junctional current traces recorded from a cell pair expressing wild-type Cx43. The intracellular pipette solution contained 0.1 mmol/L of a synthetic peptide (L2) corresponding to sequence 119 to 144 of Cx43. Recordings were done in the presence of 0.5 mmol/L octanol (Vj=-60 mV). A, Three single channel transitions between closed (C) and open (O) states. Notice the absence of a residual state. Unitary conductance, measured from the all-points histogram, was 114.8 pS. B, Transitions from closed to 2 conductive states (O1 and O2) and no residual state. Unitary conductance: 112 pS.
of the residual state, the all-events histogram of Cx43 channels in the presence of the L2 peptide was best described by a single Gaussian function centered at 99.0±0.5 pS (Figure 2). The mean unitary conductance was similar to that reported previously for the wild-type channel, thus suggesting that the peptide does not modify the unitary conductance of the channel in the open state.

The L2 Region and the Residual State

The results noted above are consistent with the hypothesis that the residual state is consequent to the interaction of the L2 region with a separate domain of Cx43, which acts as a gating particle. Accordingly, the free peptide would bind the gating element and prevent its interaction with the native L2 domain. This model would imply that a mutation in the L2 region that interferes with its binding to the gating element should also interfere with the residual state. Although preservation of a conductive channel pore is highly dependent on the integrity of the L2 region, we found a specific double mutation (H126K-I130N) that was compatible with channel function. The latter led us to propose that an L2 peptide harboring the H126K-I130N mutation would fail to interfere with the residual state.

Effect of Peptide H126K/I130N on the Biophysical Properties of Cx43 Channels

The data in Figure 3 would suggest that mutation H126K-I130N prevents the interaction between the L2 region and the CT domain. The latter led us to propose that an L2 peptide harboring the H126K-I130N mutation would fail to interfere with the residual state.

In Vitro Binding of the CT Domain to Synthetic Peptides of the L2 Region: Mirror Resonance Spectroscopy

The electrophysiological data presented above would suggest that mutation H126K-I130N prevents the L2 interaction with the gating particle. Previous data suggest that the CT domain of Cx43 may act as the gating particle (see also “Discussion”). Hence, we used MRS to test the ability of peptide H126K-I130N to bind in vitro to the CT domain and compared the results with those obtained when the CT domain was bound to the wild-type L2 peptide. Figure 3B and 3C show the results. Figure 3B depicts MRS traces obtained from addition of either the L2 peptide (black trace) or peptide H126K-I130N (gray trace) to a cuvette loaded with the recombinant CT domain. Clearly, although the L2 peptide bound effectively to the CT domain, no binding of peptide H126K-I130N to the CT was detected at pH 6.5, regardless of the concentration tested (Figure 3C).

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Figure 4 shows results from these experiments. Clearly, as shown by the traces in Figure 4A, in addition to the open and residual states, the all-events histogram of Cx43 channels in the presence of the L2 peptide was best described by a single Gaussian function centered at 99.0±0.5 pS (Figure 2). The mean unitary conductance was similar to that reported previously for the wild-type channel, thus suggesting that the peptide does not modify the unitary conductance of the channel in the open state.
closed states a conductive “residual” state was recorded. The frequency of transitions into the residual state measured from these experiments was $66.3 \pm 7.8\%$ (N=6). This behavior was similar to that observed in the wild-type channel.1,2 Consistent with this observation, the histogram of events showed 2 peaks, corresponding to the transitions between residual and open ($80.3 \pm 0.4$ pS) and between closed and open ($101.7 \pm 0.5$ pS).

**Effects of L2 Peptide on Rate of Transition and Mean Open Time**

A possible explanation for the results in Figures 1 and 2 is that the L2 peptide binds to the gating particle, thus preventing its interaction with the native receptor. If the L2 peptide interfered with gating by binding to the CT domain, then changes in function induced by the L2 peptide would be similar to those observed after truncation of the CT domain. Previous studies have shown that, in the case of L2 peptide exposure, CT truncation eliminated the residual state. The same truncation also caused slowing of the rate of transition between states.5 Here, we aimed to determine whether similar effects were detected when the L2 peptide was dialyzed in the intracellular space.

**Rate of Closed–Open Transition**

A particle–receptor interaction has been proposed to be responsible for fast Vj gating. Hence, as in the case of CT truncation, interference with this interaction would be expected to prolong the rate of transition between states. An example is shown in Figure 5A. Two traces are shown. The one labeled “L2” was obtained from a cell pair where the L2 peptide was present in the pipette solution. The trace labeled “H126K-I130N” was obtained in the presence of the peptide harboring the mutation. Notice that in the case of the cell pair exposed to the L2 peptide, the channel transited between the closed and the open states, whereas in the presence of the mutant peptide, the transition originated from the residual state (see Figures 1 and 4). For these experiments, data were filtered at a cutoff frequency of 1 kHz to avoid filtering artifacts. As shown by the histograms in Figure 5C, almost all of the transitions (91.8%) recorded in the presence of peptide H126K-I130N were equal to or shorter than 3 ms. The latter is similar to the behavior observed in the wild-type channel in the absence of any peptides.2,5 In contrast, 63.9% of all measured transitions recorded in the presence of the L2 peptide required >3 ms to complete (Figure 5B). The latter suggests that the L2 peptide is able to interfere with the mechanism controlling fast Vj gating.

**Mean Open Time and Closed Time**

Previous studies have shown that the time the Cx43 channel resides in the open state is prolonged after truncation of the CT domain.5 Here, we characterized this parameter from Cx43 channels when in the presence of the L2 peptide. Only data obtained in the absence of any uncouplers and when a single channel was active in the recording were included in the analysis. Given the limited likelihood of obtaining the proper conditions for these recordings, and the clear indication that the H126K-I30N peptide was not affecting the properties of the wild-type Cx43 channel (see Figure 4), we decided to compare our results with those obtained previously from wild-type Cx43 and from the mutant of Cx43 lacking the CT domain.1,2,5,7 Figure 6A shows a histogram indicating the frequency of events that presented a given open time. A bell-shaped distribution was observed, with a mean value of 937.8 ms (N=2, n=205). This value of mean open time falls within that reported previously for wild-type channels (440 to 560 ms; Brink et al14) and that observed after truncation of the CT domain (2450 ms).5 Finally, closed times for Cx43 channels in the presence of L2 were best described by 3.
mV/H11002 values; $G_{j_{min}}$ recorded value of $G_{j_{min}}$ was lower than that reported previously for negative $V_j$ values; $V_0 = 60.9 \pm 0.1$ mV, $A = 0.08 \pm 0.001$ mV$^{-1}$, $G_{j_{min}} = 0.13 \pm 0.003$ for negative $V_j$ values; $V_0 = 65.9 \pm 0.3$ mV, $A = 0.07 \pm 0.001$ mV$^{-1}$, $G_{j_{min}} = 0.09 \pm 0.007$ for positive $V_j$ values ($N = 6$). The recorded value of $G_{j_{min}}$ was lower than that reported previously for Cx43 wild-type channels ($G_{j_{min}} = 0.35$ for negative $V_j$ values; $G_{j_{min}} = 0.39$ for positive $V_j$ values) and similar to that observed after truncation of the CT domain ($G_{j_{min}} = 0.08$ for negative $V_j$ values; $G_{j_{min}} = 0.09$ for positive $V_j$ values). These data are consistent with the notion that the L2 peptide interferes with gating to the residual state of the channel.

Figure 6. Effect of L2 peptide on dwell open time and closed time of Cx43. Data were obtained from cell pairs expressing wild-type Cx43 in the presence of 0.1 mmol/L L2 peptide in intracellular pipette solution. Only 1 channel was active at time of recording. Mean open time was 937 ms. Closed-time histogram was best described by 3 Gaussian functions centered at 14.8 (major peak) and 668.8 and 101612.4 ms (minor peaks).

### Voltage Dependence of Cx43 Channels in the Presence of the L2 Peptide

Intracellular delivery of the L2 peptide led to a modification of the voltage dependence of Cx43 channels. Results are presented in Figure 7. The plot depicts the junctional conductance as a function of transjunctional voltage. The characteristic bell-shaped function was recorded. The data were best fit by a Boltzmann function with the following parameters: $V_0 = 60.9 \pm 0.1$ mV, $A = 0.08 \pm 0.001$ mV$^{-1}$, $G_{j_{min}} = 0.13 \pm 0.003$ for negative $V_j$ values; $V_0 = 65.9 \pm 0.3$ mV, $A = 0.07 \pm 0.001$ mV$^{-1}$, $G_{j_{min}} = 0.09 \pm 0.007$ for positive $V_j$ values ($N = 6$). The recorded value of $G_{j_{min}}$ was lower than that reported previously for Cx43 wild-type channels ($G_{j_{min}} = 0.35$ for negative $V_j$ values; $G_{j_{min}} = 0.39$ for positive $V_j$ values) and similar to that observed after truncation of the CT domain ($G_{j_{min}} = 0.08$ for negative $V_j$ values; $G_{j_{min}} = 0.09$ for positive $V_j$ values). These data are consistent with the notion that the L2 peptide interferes with gating to the residual state of the channel.

Figure 7. Effect of L2 peptide on the voltage dependence of Cx43. Data obtained from 6 cell pairs expressing Cx43. Junctional currents were recorded during a 400 ms/mV voltage ramp. The resulting data were averaged and fitted by a Boltzmann equation. Intracellular pipette solution contained 0.1 mmol/L of L2 peptide. Recorded $G_{j_{min}}$ values were 0.13 $\pm$ 0.003 (at negative $V_j$) and 0.09 $\pm$ 0.007 (at positive $V_j$). These values were similar to those observed after truncation of the CT domain.

### Discussion

We have characterized the effect of a peptide of the L2 region on Cx43 channel function. We show that this peptide causes the following: (1) a decrease in the predominance of transitions to the residual state without modification of the main open state conductance, (2) prolongation of open time and rate of transitions; and (3) reduction in $G_{j_{min}}$. These results correlate with the ability of the peptide to bind to the CT domain, as determined by MRS. The changes in channel function caused by the presence of the L2 peptide are analogous to those obtained when the CT domain is truncated from the sequence. Overall, the data support the hypothesis that the L2 region acts as a "receptor" that interacts with a flexible intracellular gating element (probably including the CT domain) to bring about the residual state.

### Technical Considerations

Although the results obtained from patch clamp studies are consistent with the in vitro MRS data (Figure 3B and 3C), we recognize that the behavior of a recombinant protein in solution may differ from that of the native protein in the cellular environment. This concern applies to all in vitro studies, particularly those in which protein structures are solved, given the extreme conditions necessary for protein isolation, ordering, and/or crystallization (eg, Unger et al19). Particularly worth noting is the fact that MRS experiments were conducted at pH 6.5; indeed, lowering the pH of the solvent significantly increases the structural order of L2 and its binding affinity for CT. Yet, the electrophysiological experiments were conducted at normal pH. Whether the cellular environment facilitates the organization of the structure even at the normal pH of the cell is likely, but remains to be determined.

We delivered synthetic peptides via the patch pipette to determine their effect on channel properties. Yet, it is always possible that the peptide fails to reach the area of cell–cell apposition and/or that it gets degraded by intracellular proteases. To increase the chance that the peptide had reached the gap junction plaque, we measured only channel activity acquired 30 minutes after patch break. The decrease in the frequency of transitions into the residual state suggests that the L2 peptide was indeed active in the cell. Although we cannot be certain of the same with regard to peptide H126K-I130N, it seems unlikely that the mutation affected the ability of the peptide to diffuse into the cell or increased its degradation rate by proteases. Rather, we propose that peptide H126K-I130N did not interfere with the residual state because it failed to interact with the gating particle.

### The L2 Peptide as a Competitive Inhibitor of Gating

It is our hypothesis that the L2 region acts as a receptor for the gating particle. Accordingly, it should be possible to use site-directed mutagenesis to prevent gating either by deletion of the gating particle (likely the CT domain; see Revilla et al7 or Moreno et al18) or by deletion of the L2 region. However, deletions within the L2 region render the Cx43 protein unable to form conductive channels.13 We found a particular double mutation (H126K/I130N) that allowed us to observe func-
tional gap junction channels, though at a very low probability. Our recordings revealed a drastic reduction of the frequency of transition into the residual state for this particular mutant. Based on that information, we tested a pair of synthetic peptides of the L2 region. We reasoned that if the L2 region were the receptor, the corresponding peptide would bind to the gating particle and prevent it from binding to the native receptor. A peptide bearing the H126K-I130N would not bind to the particle, and, therefore, channel function would be unaffected. The end result (prevention of the particle-receptor interaction by the L2 peptide) would then be analogous to that obtained after deletion of the gating particle. The results in this article are consistent with this hypothesis.

**Effect of the L2 Peptide in Comparison With Truncation of the CT Domain**

Although our results present similarities with those obtained after truncation of the CT domain, important differences need to be considered. In particular, the estimated value of mean open time in the presence of the L2 peptide (937 ms; see Figure 6A) is less than what Moreno et al reported for the truncated channel (2450 ms). It is worth noting that both of these values are, in turn, significantly higher than what had been reported for wild-type Cx43. Yet, the prolongation of open time is more pronounced after CT truncation. Similarly, our data show a 5- to 10-mV increase in \( V_0 \) in the presence of the L2 peptide. The latter seems consistent with the solved structure of the Cx43 channel. Indeed, the data show electron densities in the intracellular region that are continuations of 1 of the pore-forming transmembrane domains, likely TM3. These densities are consistent with \( \alpha \)-helical configurations (as proposed) and their position with respect to the rest of the channel would place them as a part of the pore vestibule. Further studies will be necessary to definitely assign a correlation between primary sequence of Cx43 and the high-order structure of the channel.

**Position of the Gating Particle in the Cx43 Sequence**

Studies conducted in Cx32 channels, led to the conclusion that the gating element corresponds to the amino terminal (NT) domain of the protein. By inference from those studies, it was proposed that the NT domain is also the gating particle of Cx43. Yet, as noted by Harris, this extrapolation is questionable, given that there are important differences in the primary sequences of the NT domains, thus making the structural model developed for Cx32 not directly applicable to Cx43. In the absence of experimental confirmation, other structural models should be considered.

We have proposed that amino acids within the CT domain may act as a gating particle. This hypothesis is supported by the following observations: (1) fast \( V_0 \) gating is eliminated after truncation of the CT domain; (2) truncation of the CT domain also eliminates the residual state; (3) the residual state is restored if the CT domain is coexpressed as a separate particle. Here we further show that a peptide that binds to the CT domain (see Duffy et al; see also Figure 3B and 3C) mimics the functional consequences of CT deletion. Although our data do not discard the possible participation of other regions of the protein in the gating process, it points to the CT domain as one of the components that interact with the channel vestibule to bring about the residual state.

**The CT-L2 Binding: A Particle–Receptor Interaction**

We propose that the L2 region is a part of the channel vestibule and that the CT domain is part of the gating particle.
Evidence that these 2 domains are able to bind to each other comes from our spectroscopic studies. Here, we have shown that a peptide of the L2 region modifies channel function in a manner consistent with what would be predicted if the L2 region acts as a receptor for the gating particle. These data show that small peptides can be used to interfere with gap junction function, as predicted by structural analysis. Our results may lead to the development of a gap junction pharmacology based on rational drug design.

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References
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