Extracellular Surface Charges in Voltage-Gated Ion Channels

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A large number of charged amino acids are in the extracellularly located parts of the voltage-gated ion channels. Recent findings suggest that these surface charges contribute to the channel functions in the sensing of voltage, the binding of substances, and the sensing of H+ concentration.

Voltage-gated ion channels play important roles in the functioning of several cell types. Ion channels have thus become one of the major objects in physiological research. Although the function of ion channels has been under investigation for a few decades, only in recent years has insight into the structural properties of voltage-gated ion channels been added (cf. Ref. 11). Although the complete three-dimensional structure of the voltage-gated ion channel is still unknown, the structure of the pore region has been determined by X-ray crystallography of a bacterial potassium channel (2), and topological models have been developed on the basis of hydrophobicity profiles and mutagenesis experiments (Fig. 1). The channel—or at least the central part carrying the pore and the voltage sensor—is composed of four basic structural elements that are termed subunits, domains, or repeats (Fig. 1A). Each element consists of an amino acid chain that is thought to have six putative α-helical membrane-spanning segments, termed S1 to S6 (Fig. 1, B and C). The amino and carboxy termini of the amino acid chain face the cell interior. The linkers connecting the segments S1 with S2, S3 with S4, and, in part, S5 with S6 are assumed to be located extracellularly and to form, at least for the most part, the extracellular surface of the voltage-gated ion channels.

For several regions of the ion channel molecule the functional role has been predicted. Experimental studies have especially focused on the central part of the region between segments S5 and S6, which forms a major part of the ion conducting pathway (p region or pore; Ref. 2), whereas the S4 segment has been shown to comprise at least part of the voltage sensor (cf. Ref. 12). Although a large number of studies have dealt with the structure-function relation of these regions, comparably little attention has been paid to the structure-function relation of the extracellularly located linkers of the channel molecule. The charged amino acids of these regions are of particular interest because they contribute to an extracellular surface charge, which might affect the process of voltage gating and binding of extracellularly applied substances and protons.

In this article, the possible function of the charged amino acids in the extracellular linkers is summarized and discussed. Thus attention is focused on the glutamate and aspartate
residues, which are negatively charged, whereas the lysine, arginine, and, in part, histidine residues carry a positive charge. Besides the charged amino acids, however, in some investigations sialic residues attached to asparagine-linked oligosaccharide chains have been suggested to contribute to the surface charges. The contribution of the sialic residues is not yet clear, and they are not considered in this article because 1) in an investigation on five potassium channel types there was no correlation between the estimated charge density and the number of putative glycosylation sites (3); 2) removal of the respective amino acids by site-directed mutagenesis showed only negligible effects on the voltage dependence of activation and steady-state inactivation of potassium channels (cf. Ref. 3); and 3) no significant changes in potassium channel activation were found after prevention of glycosylation by tunicamycin (unpublished observations).

Sensing of voltage

It has been known for several decades that an increase in the extracellular concentration of divalent cations changes the voltage dependence of channel activation and shifts the activation curve to positive potentials (cf. Ref. 7). The most likely explanation for this phenomenon is a change of the electrical potential at the extracellularly facing surface of the ion channels. This change in surface potential is assumed to affect the voltage difference between the extra- and intracellular compartment, which is measured by the voltage sensor within the membrane (cf. Ref. 12). In detail, the extracellularly applied divalent cations make the extracellular potential more positive and thus increase the transmembrane potential difference measured by the voltage sensor of the channel. Therefore, a greater depolarization is needed to reach the threshold potential of channel activation and the activation curve is shifted to more positive potentials (cf. Ref. 7). Thus the explanation suggests that the extracellular surface charges in general are involved in the process of voltage-dependent channel activation.

Concerning the charged amino acids, measurements on five cloned potassium channels have shown a correlation between the midpoint potentials of the activation curves and the calculated charge densities obtained from the net charges of amino acids as derived from the primary structures (Fig. 2; cf. Ref. 3). The results suggest that the charge of extracellularly located amino acids contributes significantly to the potentials of channel activation and that, e.g., a large number of negatively charged amino acids in the extracellular linkers induces channel activation already at comparatively negative potentials (3).

In line with this hypothesis is the finding that in the eag potassium channel, the substitution of an uncharged amino acid by the partly positively charged histidine (L342H; Fig. 3A)
shifted the midpoint of activation for a few millivolts in a positive direction (15). A shift of −50 mV in the hyperpolarizing direction was obtained by inducing a negative charge in the linker near the S4 segment (A345E; Fig. 3A; Ref. 15). However, this effect is most likely not caused by the change in surface charge but rather in conformational stability, because a positively charged amino acid at this position had the same effect (A345R; Ref. 15). Furthermore, the deletion of a negatively charged portion (ΔDRDED333-337; Fig. 3A) of the linker between S3 and S4 shifted the midpoint of activation of the eag channel in the positive direction (15). In contrast, the deletion of three negatively charged amino acids in the same position in the Shaker channel (ΔEEE333-335; Fig. 3A; Ref. 10) had the opposite effect. The substitution of the four negatively charged amino acids at this position by positive residues (EEED333-336KKKK; Fig. 3A; Ref. 10), however, induced a small positive shift of the activation curve. Finally, the insertion of a slightly negative amino acid chain in the S3-S4 linker of the eag channel (27 additional residues, additional net charge −1; Ref. 4) caused a small negative shift of the activation curve.

In summary, most experiments of site-directed mutagenesis have shown only small and inconsistent effects on channel activation curves after changes of charged amino acids. However, it must be considered that changes in length or sequence are likely to alter not only the charge but also the conformation of the linker, thus possibly influencing the process of gating. Furthermore, point mutations of single charged amino acids will induce only small changes in the surface charge, and thus it is very likely that the single changes have only negligible effects on the activation curves. Additionally, the studies have been focused on the S3-S4 linker; comparable site-directed mutagenesis experiments on the other linkers are still lacking. It might be possible that other linkers are of greater importance for the voltage-dependent activation processes. Thus it is assumed that strontium—which is suggested to screen mainly surface charges with little or no binding—shifts the activation curve of voltage-gated potassium channels by acting on the charged amino acids in the linker between the S5 segment and the pore region (3). In conclusion, the existing investigations do not yield decisive evidence, and it remains unclear whether the charged amino acids of the extracellular linkers are of relevance for the voltage dependence of channel activation.

### Binding of substances

Several studies have shown that charged amino acids are involved in forming the binding sites for extracellularly applied substances. Thus the sensitivity of the potassium channel Kv1.1 to the peptide α-dendrotoxin was reduced 30-fold when a glutamate residue was substituted by serine in the linker connecting the S5 segment with the pore region (E353S; Fig. 3B; Ref. 9). Correspondingly, the converse mutation in the dendrotoxin-sensitive potassium channel Kv1.3 induced a 10-fold higher sensitivity to dendrotoxin (S375E; Fig. 3B; Ref. 9). In the same linker of the Shaker potassium channel, the change of an aspartate residue to a positively charged amino acid (D431K; Fig. 3B) made the channel insensitive to charybdotoxin (5), whereas the opposite change in charge at another position of the linker (K427D; Fig. 3B) increased the sensitivity >50-fold (5). For the sensitivity to the charybdotoxin isoform agitoxin, two charged residues were identified in the linkers connecting S5 with the pore region and in the successive extracellular linker from the pore to the sixth segment of the potassium channel. Mutations of the two lysine residues to uncharged amino acids (K356G and K382V; Fig. 3B) increased the sensitivity of the Kv2.1 channel to agitoxin, >2,000-fold (6).

In the linker S3-S4 in domain IV of the type IIa sodium channel, changes of a glutamate residue to a positively charged one (E1613R; Fig. 3B) increased the affinity for α-scorpion toxin and sea anemone toxin >60-fold. Also, the substitution of the positively charged amino acid lysine by a neutral one (K1617A; Fig. 3B) enhanced the binding of the two toxins (13). Furthermore, the binding of sea anemone toxin was significantly affected by a glutamate in the same linker (E1616R; Fig. 3B; Ref. 13). For binding of the potassium-channel blocker hanatoxin to the Kv2.1 channel, charged residues in the linker between segments S3 and S4 are also involved. Thus substitution of glutamate (E277A; Fig. 3B) or arginine (R290A; Fig. 3B) in this linker decreased the sensitivity of the channel to hanatoxin 15-fold and 5-fold, respectively (14).

The above findings indicate that charged amino acids in the extracellular linkers play an important role in the binding of several substances. However, in all of these reported investigations, changes in the sensitivity of the channel to the respective substances could also be induced by site-directed mutagenesis of uncharged amino acids in other positions of the involved linker. This indicates that, besides the position and number of defined charges, conformational changes in the linkers are also involved in the formation of the binding site for several extracellularly acting substances.

![Figure 2](https://example.com/figure2.png)
Sensing of H\(^+\) concentration

Although the charge of glutamate and aspartate as well as that of lysine and arginine are barely affected at physiological pH values, the isoelectrical point of the amino acid histidine is at a pH value of 7.6. Therefore, it can be assumed that histidine is partially charged at physiological pH values. Furthermore, changes in H\(^+\) concentration will change the protonation and thus the charge of this amino acid. Because of this characteristic, it is assumed that histidine residues might be involved in sensing H\(^+\) concentration ([H\(^+\)]) or in [H\(^+\)] modulation of voltage-gated ion channels. Indeed, this idea has been confirmed for the voltage-gated, inwardly rectifying potassium channel KST1 of guard cell protoplasts (8). Extracellular acidification shifted the voltage dependence of channel activation to less negative potentials, thus increasing the potassium current at a fixed potential. Mutational analysis demonstrated a relationship between this acid activation and two extracellular histidines. The pH sensitivity of the potassium channel was weakened when the histidine in the extracellular linker between S3 and S4 was substituted (H160A; Fig. 3C; Ref. 8). The pH sensitivity was even more affected when the histidine in the linker between the pore region and the S6 segment (cf. Ref. 2) was changed to an uncharged amino acid (H271A; Fig. 3C; Ref. 8). Furthermore, the substitution of both histidines by alanine led to a complete loss of the channel's pH dependence (8).

A pH-dependent change of potassium channel conductance was reported for the potassium channel HIR (1). For this channel, an increase in single-channel conductance was associated with an increase in external pH. The molecular determinant was localized to a histidine residue in the extracellular linker immediately above the pore region (H117; Fig. 3C; Ref. 1). Although this study has been conducted on an inward rectifier potassium channel, the result might be of importance for voltage-gated ion channels, because several potassium channels have histidine residues at corresponding positions (cf. Ref. 11). Thus, for the Kv1.1 channel there is preliminary evidence that this histidine is responsible for the pH dependence of the tetraethylammonium (TEA) blockade because the exchange of the histidine by the uncharged glycine (H355G; Fig. 3C) made the TEA effect independent of pH [Bretscher et al. (abstract), Biophys. J. 74: A116, 1998].

In summary, evidence is growing that the charge of the histidine residues in the extracellular linkers—and especially the change of this charge by different proton concentrations—is involved in the pH-dependent modulation of activities and properties of voltage-gated ion channels. However, it must be taken into account that the acidic “microclimate” surrounding cells is affected by the surface charge effects of the glycocalyx and by the membrane phospholipid head groups themselves. Therefore, the pH on the extracellular surface of the cell membrane can be considerably below that of the bulk solution. Thus it cannot be excluded that the charges of other amino acid residues are also changed by protonation and are contributing to the sensing of [H\(^+\)].
In this review, the results concerning the role of charged amino acids in the extracellularly located parts of voltage-gated ion channels have been summarized. Although the findings offer support for a role of the surface charges for channel function and properties, the absolute relevance—especially for the function of voltage sensing—is not yet clear. Thus the roles of several charged amino acids and of large parts of the channels' extracellular surface (e.g., the linker between segments S1 and S2) have not been experimentally examined. Therefore, this article can only hint at the possibility of the great importance of extracellular surface charges for the function and properties of voltage-gated ion channels.

References