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Gating charge transfer due to fixed ionizable sites

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Abstract. An alternative origin is suggested for one component of the gating charge transfer that is measured just prior to and during the opening of an ion channel. Rather than it originating solely from the motion of groups with fixed charge, some may stem from ionizable sites which remain fixed in position but change their state of charge. Such changes involve proton migration across the membrane. Two cases are discussed. In the first the ionizable group changes its charge in response to the change in its dielectric environment resulting from the formation of an aqueous pore and in the second the change is as the direct result of the applied trans-membrane voltage. Some of the predicted characteristics of this novel component of gating charge transfer are described.

Key words: Gating charge – Ion channel – Fixed ionizable site

Introduction

When attempting to understand the operation of voltage-gated ion channels one of the most important measurements is that of gating charge transfer. The passage of ions through the channel is deliberately blocked but the change in the electrical structure of the channel protein prior to and during the opening of the channel is measured. By studying the voltage and time dependence of this change it is hoped to shed light on the detailed operation of the ion channel as described in detail in two reviews by Armstrong (1981) and Keynes (1983). Hitherto it has been assumed that the measured change in electrical structure is due to groups bearing a fixed charge moving across the membrane or equivalently to electric dipoles rotating within the membrane. Here it is shown that a significant contribution to the measured gating charge transfer may be due to ionizable residues which remain fixed in position but which change their state of charge, either in response to changes in the electrical polarizability of parts of the channel protein when the channel opens, or directly to the effects of the trans-membrane voltage.

Gating charge transfer when the channel opens

Osmotic stress experiments show that when the potassium channel of the squid axon opens an aqueous pore volume of about 1300 $Å^3$ is created (Bezanilla et al. 1986) and for the mitochondrial voltage dependent anion channel the volume is $2–4 \cdot 10^4$ $Å^3$ (Zimmerberg and Parsegian 1986). The intrusion of such a large volume of highly polarizable aqueous pore will cause substantial changes in the effective pK values of ionizable sites near the pore, increasing the probability that they become charged. Detailed examples of such effects have been given in a recent paper (Edmonds 1989) which show that increases in the effective pK of basic ionizable sites and decreases in the effective pK of acid ionizable sites of 4 units or more are possible when the pore is created. The essential mechanism is that the electric field of a charge induces electric dipoles in a neighbouring polarizable region in such a direction as always to reduce the magnitude of the electric potential at the surface of the charge. This is true for both positive and negative charges. Lowering the magnitude of the electric potential at its surface when it is charged, increases the probability that an ionizable site will be charged, whether it be an acid site that becomes negatively charged by losing a proton or a basic site that becomes positively charged by aquiring a proton.

In the conventional experiment to measure gating charge transfer across the membrane of an axon, described in the reviews quoted above, the inside of an axon is held at a fixed voltage relative to the fluid bathing the axon by means of a voltage clamp. The gating charge transfer measured in such an experiment is defined as positive if it would result from a positively charged group moving outward across the membrane. Although the ionizable site is assumed fixed in position, the change of its charged state requires the migration of a proton between the aqueous fluid bathing one face of the membrane and the fixed site. The gating charge transfer measured will be positive if the proton migrates outward across the membrane and negative if it migrates inward. Thus an acid site that becomes negatively charged by losing a proton will

give rise to positive gating charge transfer if the proton migrates to the outer fluid bathing the axon and to negative gating charge transfer if the proton migrates to the inner fluid. A basic ionizable site which becomes positive on binding a proton will give rise to positive gating charge transfer if the proton comes from the inner fluid and negative gating charge transfer if the proton comes from the outer fluid. The magnitude of the gating charge transfer will be $q \cdot x/t$ when the proton migrates a distance x perpendicularly across a membrane of electrical thickness t, where q is the proton charge.

Some of the predicted characteristics of this type of gating charge transfer are listed below.

(i) As discussed above it may of either sign.
(ii) For sites close to the pore, proton kinetics are fast (Prod'hom et al. 1987) so that it will appear quantal in nature.
(iii) Changing the pH of the fluids bathing the membrane faces can change the amount of gating charge transfer. Over a central range of pH values, such that the change in the site pK caused by pore formation leads to a change in the charged state of the site, the magnitude of the gating charge transfer will depend little on external pH. However outside this range at lower and higher pH the magnitude of the gating charge transfer will decrease sharply.
(iv) If the creation of the aqueous pore is a sudden single event then both statically as a function of membrane voltage and kinetically as a function of time, this component of the gating charge will mirror channel opening. Its onset will be delayed as is the conductance change of an assembly of channels, which will lead to a rising phase of the measured gating current. If however the aqueous pore is created in a series of steps then this component of gating charge transfer may itself proceed in steps, most of which precede channel opening.
(v) If inactivation is assumed to be due to a charged plug being attracted into the inner mouth of the channel, blocking the channel, but maintaining the presence of the aqueous pore, (Armstrong and Bezenilla 1977; Edmonds 1983), then much of this type of gating charge will be immobilized by inactivation.

There is ample evidence that part of the measured gating charge is immobilized on inactivation of the channel conductance (Armstrong and Bezanilla 1977; Keynes 1983). It is also well established that changes in pH effect measured gating charge transfer. Campbell and Hahin (1984) found complex changes in the gating charge observed in frog skeletal muscle fibres when the external pH was lowered to 5 which were not accounted for by a shift along the voltage axis. Wanke et al. (1983), using Squid giant axons, found that high internal pH markedly changed the fraction of the gating charge immobilized and simultaneously increased the total amount of gating charge detected.

Charge transfer due to fixed sites in the membrane field

Because a change in the state of charge of the ionizable site involves the migration of a proton across part of the membrane, it will also respond directly to the membrane voltage. Such effects may be illustrated using the simplified model system shown in Fig. 1 a which consists of two basic ionizable sites symmetrically placed within a membrane of dielectric constant ε_2 bathed by a fluid with dielectric constant ε_1. Each site has the same effective pK (pKe) when the other is uncharged but the sites interact electrostatically such that if one is charged the positive voltage at the other greatly reduces the probability that the second is charged. In this planar geometry the field at one site due to the other may be calculated using the method of images (Neumcke and Läuger 1969). We will assume that each site exchanges protons only with the aqueous fluid on its side of the membrane. A positive voltage between the top (inner) surface of the membrane and the bottom will increase the probability of transfer of a proton from the upper fluid to the upper site. The voltage difference ΔV between the site and the proton fluid is negative which increases the probability of the site being charged. Similarly the same positive voltage will decrease the probability that the lower site is charged as now the voltage difference ΔV is positive between this site and the neighbouring (outer) aqueous fluid. A calculation of the gating charge transfer that would be measured as a function of the trans-membrane voltage for such a geometry can then be simply computed using a Monte Carlo calculation.

At each tick of a clock one of the two sites is selected randomly. Knowing the charge state of both sites and the membrane voltage the voltage difference between this site and the fluid bathing the nearest face of the membrane is determined. Knowing this the probability that the site is

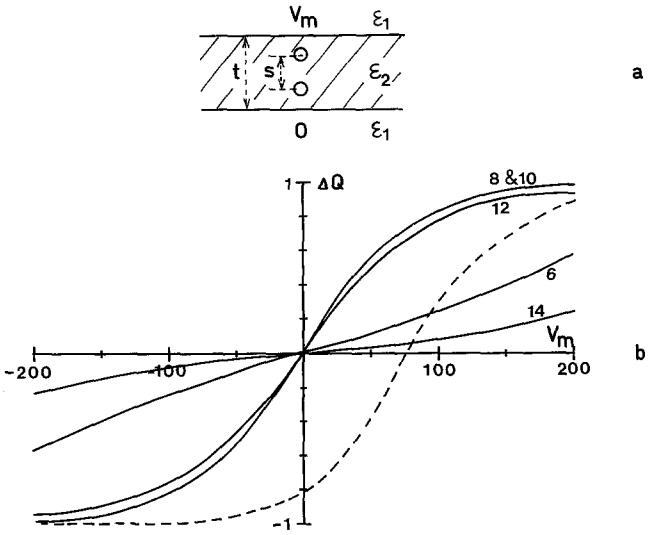

Fig. 1. **a** Two identical ionizable sites separated by a distance s and symmetrically positioned in an infinite parallel-sided solid slab of dielectric constant ε_2; The slab has thickness t and is bathed on both faces by aqueous fluid of dielectric constant ε_1 and controlled pH. **b** The extra charge ΔQ that has to be applied to the upper conducting fluid to maintain its voltage at V_m when the ionizable sites change their state of charge in response to the applied ovltage V_m measured in mV. ΔQ is measured in units of $q(t-s)/2$ where q is the proton charge. The parameters chosen and the details of the calculation are given in the text

charged is then determined. A comparison between this probability and the output of a random number generator determines the state of charge of this site at this time. By repeating this procedure the system is allowed to reach equilibrium, after which the time average of the state of charge of the two sites is determined over a further million clock ticks. In this manner, the equilibrium state of gating charge transfer may be obtained at each membrane voltage. Fig. 1 b shows the computed charge transfer ΔQ as a function of the membrane voltage in millivolts, for various values of pKe. The values of the parameters chosen were $\varepsilon_2 = 5$, $\varepsilon_1 = 80$, $s = 10$ Å, $t = 30$ Å and the fluid pH = 7.

At large positive membrane voltages the upper site is charged and the lower is uncharged whereas at large negative voltages the lower is charged but the upper is not. At intermediate voltages the usual sigmoid type of gating charge transfer characteristic is seen. For the parameters chosen the characteristic is approximately constant for pKe values between 8 and 12 but the charge transfer is reduced at lower pKe, when the probability of either site being charged is very small, and at higher pKe, when the influence of the membrane voltage is small and both sites are equally likely to be charged. One difference from the usual situation, when a similarly shaped sigmoid curve is predicted due to the motion within the membrane of fixed charges, is that the maximum slope of the characteristic depends on the pH.

The broken curve is calculated with all parameters as before but with $pH_{out} = 6$, $pH_{in} = 7$ and $pKe = 10$. The lowering of the outer pH is seen to shift the characteristic along the voltage axis to higher voltages. Such shifts are observed experimentally (Campbell and Hahin 1984) but are usually attributed to the partial neutralization of negative charges on the outer surface of the membrane by the extra protons in the outer fluid. The model discussed here suggests an alternative explanation for part of the observed shift.

Conclusion

The possibility that part of the experimentally measured gating charge transfer may be due to fixed ionizable sites has been discussed for two cases. In the first it is due to dielectric changes in the channel protein when the channel opens and in the second to the direct influence of the membrane voltage. In practice it is likely that a composite effect will occur with ionizable sites changing their charge under the joint influence of dielectric changes and the membrane voltage simultaneously.

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Note added in proof

Direct experimental evidence of a component of the gating charge which has the characteristics described in this paper is presented in Keynes and Forster (1990). In particular this component of gating current exhibits a rising phase, experiences a delay similar to the opening of the channel and is abolished by inactivation of the channel just prior to the measurement.

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