AVERAGE ELECTROSTATIC POTENTIAL BETWEEN THE FILAMENTS IN STRIATED MUSCLE AND ITS RELATION TO A SIMPLE DONNAN POTENTIAL

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ABSTRACT An approximate analytical solution to the Poisson-Boltzmann equation for a cylindrical particle was used to calculate the relationship between the charge on the filaments and the average electrostatic potential. Both thick and thin filaments were considered in the muscle lattice with a filament charge ratio of 4 to 1. Comparing this with a similar relationship obtained using simple Donnan theory showed a discrepancy at high charge where the Poisson-Boltzmann equation leads to saturation of the average potential. However, using two separate experiments from the literature, we have shown that at pH 7.0 muscle must not be close to saturation and thus is in a region of the curve where the two approaches agree.

INTRODUCTION

The filaments in muscle are charged at physiologic pH because of the presence of the ionizable groups on the thick and thin filament proteins. In modified muscle systems where the membrane properties have been destroyed by either glycerination or skinning, several laboratories (Collins and Edwards [1971], Pemrick and Edwards [1974], Elliott et al. [1978], Bartels and Elliott [1981], and Stephenson et al. [1981]), have measured and characterized a tissue potential between the muscle and its bathing solution using microelectrodes. This tissue potential has been associated with the fixed electrostatic charges on the filaments and has been shown to display the characteristics of a simple Donnan potential.

The filament lattice spacing in muscle, however, is much larger than the Debye length, so the electrostatic potential cannot be the simple constant Donnan potential, but is a strongly varying function between any two filaments. The microelectrodes that are used to measure the tissue potential are typically 0.1–1.0 μm, compared with the filament spacing of ~40 nm. Thus it is believed that the measured tissue potential is an average of the potential well over many unit cells of the filament lattice.

The electrostatic potential is described by the Poisson-Boltzmann (P-B) equation. When the potential is small the P-B equation can be linearized and an analytic solution is possible. In this case it can be readily shown that the average potential between the filaments is equal to the Donnan potential.[1] When the potential is larger, the P-B equation cannot be linearized and there is no known analytical solution for a charged cylinder. In the present work an approximate solution of the P-B equation (Philip and Wooding, 1970) is used to calculate the average electrostatic potential as a function of the filament charge. This is compared with the solution obtained by applying simple Donnan theory. It is shown that although the two curves diverge at high charges they are in good agreement in the region applicable to muscle.

THEORY

Solution to the P-B Equation

The P-B equation for a long cylinder in a monovalent salt solution reduces in cylindrical polar coordinates to

$$r^{-1}\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}E}{\mathrm{d}r}\right)=\frac{8\pi ne}{\epsilon}\sinh\left(\frac{eE}{kT}\right)$$

where r is the radial space coordinate, ϵ is the dielectric constant of the solution, E is the potential, n is the concentration of the ions, e is the electronic charge, k is the Boltzmann constant, and T is the absolute temperature.

Philip and Wooding (1970) noted that $\sinh y = y$, $0 < y < 1$; $\sinh y = \frac{1}{2}\exp \cdot y$, $y > 1$. Using these approximations Philip and Wooding were able to solve the P-B equation, and matched the solution and its derivative at the boundary between the two regions. Their solution is given in terms of the nondimensional parameters R and ψ where $R = (8\psi ne^2/\epsilon kT)^{1/2}r = Kr$, and $\psi = eE/kT$, where K is the Debye length.

[1]Naylor, G. R. S. Unpublished results.

In the region $\psi < 1$, the solution is in terms of the Bessel function K_0 and is the common Debye-Huckel approximation; whereas in the region $\psi > 1$, there are five different solutions depending primarily on the cylinder radius. Case 5 (Philip and Wooding [1970] Fig. 5) is the solution applicable to muscle where the cylinder radii are $\gg 1.5$ nm (note K is $\simeq 1$ nm^{-1} for 0.1 M salt solution).

Philip and Wooding compared their solutions with exact machine integrations showing negligible differences (<0.2% for the cases appropriate to the present paper). Therefore, their approximate solution was used to calculate the average potential over the muscle filament lattice.

Averaging over the Muscle Lattice

The distance between the centers of the thick filaments is 20 nm, and the Debye length appropriate to an ionic strength of 0.15 (as is the case for muscle) is 1 nm^{-1}. Thus the interaction between cylinders can be ignored, and it is reasonable to use the solution for an isolated cylinder. The muscle lattice is shown in Fig. 1*a* and, due to symmetry, it is only necessary to average over the smaller area shaded in Fig. 1*b*. Fig. 1*b* also shows the six filaments that must be considered in computing the average potential.

Simple Donnan Theory

The elementary theory of a Donnan equilibrium between two coexisting homogeneous phases has been described by Overbeek (1956). It leads to the familiar Nernst equation $n_i = n_0 \exp(-zeE/kT)$, where n_i and n_0 are the inside and outside concentrations of the species n with charge z.

For a monovalent solution, and using electroneutrality in the muscle, the fixed charge concentration [Pr] is given by

$$[\mathrm{Pr}] = n_0[\exp(neE/kT) - \exp(-eE/kT)]$$
$$= 2\, n_0 \sinh(eE/kT).$$

COMPUTATIONS

In the calculations a thin filament diameter of 7.0 nm was assumed. Approximating the thick filament as a cylinder is not very easy, and it is not immediately obvious what diameter to use; the thick filament core has a diameter of

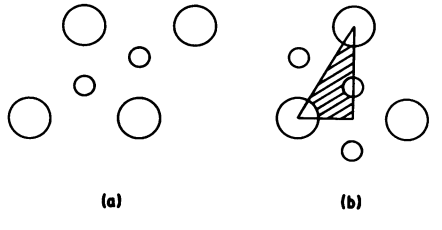

FIGURE 1 (*a*) The hexagonal cell of muscle showing the thick and thin filaments. (*b*) Part of the unit cell showing the area of integration used for the calculation of the average potential and the three thick and thin filaments considered.

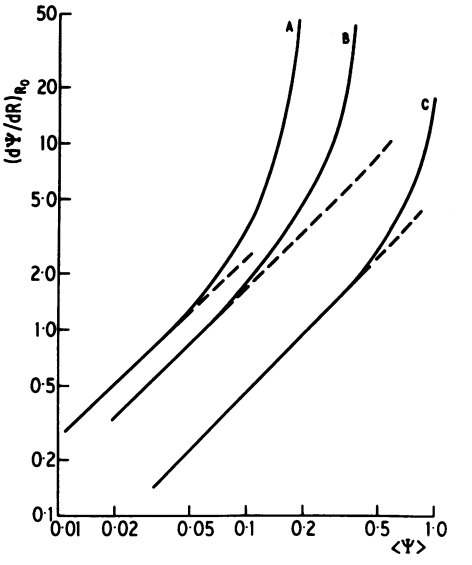

FIGURE 2 The results of the computation of the thick filament surface charge vs. the average potential. Both axes are in nondimensional units. $\langle\psi\rangle = e\langle E\rangle/kT$ and $(d\psi/dR)_{R_0} = \sigma\sqrt{(\epsilon\pi kT/2\pi)}$ where σ is the thick filament surface charge. The three curves are (*A*) thick filaments only with radius 7.5 nm, (*B*) thick filaments with radius 7.5 nm and thin filaments with radius 3.5 nm, and (*C*) thick filaments with radius 15 nm and thin filaments with radius 3.5 nm. The dotted lines are the continuation of the Donnan relationship where it disagrees with the electrostatic theory.

~15 nm, but there are cross-bridges that project a considerable distance beyond the core. Diameters of 15 and 30 nm were used. The smaller diameter is the same as that found by Millman and Nickel (1980) to be the effective diameter for pressure calculations using electrostatic theory.

Philip and Wooding's equation (1970) gives the potential and surface charge in terms of the nondimensional parameter R^*. R^* is the value of R at the boundary between the linear and nonlinear solution i.e., $\psi = 1$. It is computationally easy to calculate both for various values of R^*. It was found that step sizes of 0.1 in X and Y (the nondimensional Cartesian coordinates) were adequate for integration of the potential.

Fig. 2 shows the results of the calculations. The most prominent feature is that applying electrostatic theory there is a saturation at high charge with the average potential reaching a limiting value. This is not predicted by Donnan theory. Thus there is a forbidden region where average potentials greater than the asymptotic value are not allowed. For small potentials and charges the electrostatic theory and Donnan theory agree as can be readily shown by using a linear Debye-Huckel approximation in the P-B equation. This was used as a check for computational errors.

Another important result shown in Fig. 2 is that the deviation from Donnan theory only occurs in a relatively small region just before saturation where increasing the charge has little effect on the potential. (Millman and

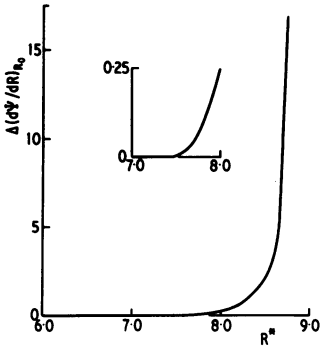

FIGURE 3 $\Delta(d\psi/dR)_{R_0}$ is the difference in the nondimensional surface charge for a given potential between the Donnan approach and the P–B equation from curve *B* in Fig. 2. It is plotted against R^* of the thick filament, the nondimensional radius at the boundary between the linear and nonlinear solutions to the P–B equation. The inset is on an expanded scale the region where the difference between the two approaches first occurs.

Nickel [1981] showed that in this region increasing the charge has little effect upon the electrostatic repulsive force). The three curves in Fig. 2 are very similar, with the main difference being that they are displaced relative to each other so that saturation occurs at different values of $\langle\psi\rangle$.

To investigate further where the deviation between the Donnan approach and the solution to the P-B equation first occurs, Fig. 3 shows the difference in the surface charge from the two approaches for a given average potential plotted as a function of R^* for the thick filament. (R^* is the nondimensional radius at the boundary between the linear and nonlinear solutions; if R^* is less than the nondimensional filament radius, the linear solution is valid in the entire region between the filaments.) The conditions for this figure correspond to curve *B* in Fig. 2; i.e., a thick filament radius of 7.5 nm and a thin filament radius of 3.5 nm. Fig. 3 shows that the two approaches agree if R^* is less than the thick filament radius and disagree for larger R^*. Thus this shows that the Donnan approach only agrees with the P-B equation in the region where it can be linearized and no further.

DISCUSSION

In glycerinated rabbit muscle at room temperature in a 0.1-M salt solution, Elliott et al. (1978) have measured potentials of -10 mV, and Bartels and Elliott (1981) have measured potentials of -5 mV; i.e., nondimensional potentials ψ are in the range of 0.2–0.4.

If 15 nm is the correct effective radius for the thick filament as Millman and Nickel (1980) concluded, then this range of nondimensional potentials ($\psi = 0.2$–0.4) is clearly in a region where the two curves for the Donnan theory and electrostatic theory coincide. However, it is possible that the curve with a thick filament diameter of 15 nm is the applicable curve. Also, if for some technical reason the measured potentials in the literature do not reflect the average potential, then the average potential could be considerably larger than that reported in the literature. These two possibilities would put muscle in a very steep part of the curve almost at saturation. Evidence from another set of experiments rules this out.

Rome (1968) measured the lattice spacing of glycerinated muscle as a function of the bathing media. Raising the pH >7.0 causes swelling of the lattice and the plot of lattice spacing vs. pH is linear from the isoelectric point pH 5.0–8.0 with only slight deviation at pH 9.0. This could be because either (*a*) all the charged groups on the proteins are ionized at pH 9.0 (see Szent-Gyorgyi [1960] for a review of the early titration curves) or (*b*) saturation of the type discussed in the present paper. These results are very strong independent evidence that at pH 7.0 the electrostatic forces are not near saturation. Taking the worst case, that pH 9.0 is already at saturation, then at pH 7.0 the spacing increase from the isoelectric point (pH 5.0) is ~60% of that at saturation. Assuming a linear attractive force opposing the electrostatic repulsion, then $\langle\psi\rangle$ at pH 7.0 should be 60% of $\langle\psi\rangle$ at saturation. From Fig. 2 this would give a discrepancy of ~20% or less between electrostatic theory and the Donnan approach.

It is interesting to note that averaging over the whole lattice to simulate the microelectrode sampling gives only a small weighting to the narrow region several Debye lengths thick around each charged filament. It is only in this limited region that the potentials are possibly large and the nonlinear solution to the P-B equation is applicable. Thus any discrepancy between the linear and nonlinear solutions in this region is reflected as a much smaller deviation in the average potential.

In conclusion, it has been shown that for muscle at pH 7.0, despite the relatively high charges and deep potential well between the filaments, the average potential calculated from the P-B equation is in good agreement with that predicted by simple Donnan theory (with maximum discrepancy of ~20%).

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REFERENCES

Bartels, E. M., and G. F. Elliott. 1981. Donnan potentials from the A- and I- bands of skeletal muscle, relaxed and in rigor. *J. Physiol. (Lond.).* 317:85P–87P.

Collins, E. W., and C. Edwards. 1971. Role of the Donnan equilibrium in the resting potentials in glycerol-extracted muscle. *Am. J. Physiol.* 221:1130–1133.

Elliott, G. F., G. R. S. Naylor, and A. E. Woolgar. 1978. Measurements of the electric charge on the contractile proteins in glycerinated rabbit psoas using microelectrode and diffraction effects. *Colston Pap.* 29:329–339.

Millman, B. M., and B. G. Nickel. 1980. Electrostatic forces in muscle and cylindrical gel systems. *Biophys. J.* 32:49–63.

Overbeek, J. T. 1956. The Donnan equilibrium. *Prog. Biophys. Biophys. Chem.* 6:58–84.

Pemrick, S. M., and C. Edwards. 1974. Differences in the charge distribution of glycerol-extracted muscle fibers in rigor, relaxation and contraction. *J. Gen. Physiol.* 64:551–567.

Philip, J. R., and R. A. Wooding. 1970. Solution of the Poisson-Boltzmann equation about a cylindrical particle. *J. Chem. Phys.* 52:953–959.

Rome, E. 1968. X-ray diffraction studies of the filament lattice of striated muscle in various bathing media. *J. Mol. Biol.* 37:331–344.

Stephenson, D. G., I. R. Wendt, and Q. C. Forrest. 1981. Non-uniform ion distributions and electrical potentials in sarcoplasmic regions of skeletal muscle fibres. *Nature (Lond.).* 289:690–692.

Szent-Gyorgyi, A. 1960. Proteins of the myofibril. *In* The Structure and Function of Muscle. G. Bourne, editor. 1st edition. Academic Press, Inc., New York, 2:1–54.