Equilibrium distribution of ions in a muscle fiber

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ABSTRACT We have developed a mathematical description of the equilibrium (Donnan) distribution of mobile ions between two phases containing fixed charges. This differs from the classical Donnan derivation by including mobile polyvalent ions such as those present in intact muscle fibers and in solutions used with skinned muscle fibers. Given the average concentrations of ionic species present in intact frog muscle, we calculate that the myofibrillar fixed charge density (−42 meq/liter cytoplasmic fluid) is in close agreement with estimates from amino acid analysis of myofibrillar proteins. As expected, with negative fixed charges in the myofibril, anions are excluded from the myofibrillar space while cations are concentrated in this space; the ratio between the average intra- and extramyofibrillar concentrations for an ion of valence n is $(1.11)^n$. This model allowed us to design a bathing solution for skinned muscle fibers in which the intramyofibrillar ion concentrations closely approximate those found in intact frog muscle cells. Our model, applied to the A- and I-bands of the sarcomere, suggests that likely differences in fixed charge densities in these regions accounts for only a small fraction of the extreme concentration of phosphocreatine observed in the I-bands of intact frog muscle (D. K. Hill 1962. *J. Physiol. (Lond.).* 164:31–50.).

INTRODUCTION

The cytoplasm of skeletal muscle contains a substantial concentration of fixed charges attributable primarily to the myofibrillar proteins (see Godt and Maughan, 1988). This will lead to a difference in concentration of mobile charges between the intra- and extramyofibrillar cytoplasmic space. The fixed charge and its associated potential may have important physiological consequences, for example, in localization within the muscle cell of charged substrates involved in energy metabolism. For instance, Hill (1962) observed that phosphocreatine (PCr) in intact frog muscle was concentrated in the I-band region of the sarcomere. Because most PCr within the cell is present as a divalent anion, it is possible that fixed charge differences within the sarcomere may lead to this localization. This bears examination since recent evidence indicates that the bulk of the freely diffusible creatine kinase is also confined to the I-band (Wegmann et al., 1987).

For skinned or glycerinated muscle fibers the ionic concentration differences between the fiber and the bathing medium have been calculated assuming a Donnan equilibrium model and uni-univalent electrolyte (Collins and Edwards, 1971; Elliott, 1973; Stephenson et al., 1981; Aldoroty and April, 1984). However, since many of the major mobile constituents in muscle cytoplasm are polyvalent (e.g., creatine phosphate and magnesium adenosine triphosphate), this simplified Donnan model only approximates the physiological condition. Therefore, we have extended the Donnan model to deal with polyvalent cations and anions.

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DESCRIPTION OF DONNAN MODEL

Consider an equilibrium distribution of ions in a system of two phases corresponding to the intra- and extramyofibrillar spaces. Only the myofibrillar phase contains fixed charges, and this leads to a potential difference between the two phases. Electroneutrality is obeyed in both phases, and mobile ions are distributed according to the Boltzmann relation (Donnan, 1911). To represent the cytoplasmic environment, the electrolytes are uni-, di-, tri-, and tetravalent anions and uni- and divalent cations. This electrolytic solution accounts for the major ionic constituents of the fluid cytoplasm of muscle (Godt and Maughan, 1988; their Table 3).

Let the tetravalent anion concentration be C^{4-}, trivalent anion concentration C^{3-}, divalent anion concentration C^{2-}, univalent anion concentration C^{-}, divalent cation concentration C^{2+}, univalent cation concentration C^{+}, and let the fixed negative charge concentration in the myofibrillar phase be X^{-}. Across the myofibrillar boun-

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dary there will be a Donnan potential U, which will be inside negative due to X^-.

From electroneutrality, outside (o) the myofibril

$$C_o^+ + 2C_o^{2+} = C_o^- + 2C_o^{2-} + 3C_o^{3-} + 4C_o^{4-} \quad (1)$$

and inside (i) the myofibril,

$$C_i^+ + 2C_i^{2+} = C_i^- + 2C_i^{2-} + 3C_i^{3-} + 4C_i^{4-} + X^-. \quad (2)$$

From the Boltzmann relation, at equilibrium

$$j_o C_o^+ = j_i C_i^+ \exp [F U/(RT)] \quad (3)$$

$$j_o C_o^{2+} = j_i C_i^{2+} \exp [+2F U/(RT)] \quad (4)$$

$$j_o C_o^- = j_i C_i^- \exp [-F U/(RT)] \quad (5)$$

$$j_o C_o^{2-} = j_i C_i^{2-} \exp [-2F U/(RT)] \quad (6)$$

$$j_o C_o^{3-} = j_i C_i^{3-} \exp [-3F U/(RT)] \quad (7)$$

$$j_o C_o^{4-} = j_i C_i^{4-} \exp [-4F U/(RT)], \quad (8)$$

where the j's are activity coefficients, F the Faraday, R the gas constant per gram mole, and T is the absolute temperature.

Activity coefficients of ions depend on the ionic strength of the surrounding environment. Under physiological conditions the ionic strength inside the myofibril is probably close (within 8%) to that outside (see Results and Discussion). Thus we can greatly simplify these calculations by assuming that the activity coefficient of an ion inside the myofibril is the same for that ion outside the myofibril; i.e., $j_i = j_o$.

Inserting Eq. 3–8 into Eq. 1 and letting:

$$j_i = j_o,$$

and

$$Y = \exp [F U/(RT)] \quad (9)$$

we have:

$$C_i^+ Y + 2C_i^{2+} Y^2 = C_i Y^{-1} + 2C_i^{2-} Y^{-2} + 3C_i^{3-} Y^{-3} + 4C_i^{4-} Y^{-4}. \quad (10)$$

Multiplying both sides of Eq. 10 by Y^4 and rearranging, one obtains:

$$a_6 Y^6 + a_5 Y^5 + a_4 Y^4 + a_3 Y^3 + a_2 Y^2 + a_1 Y + a_0 = 0, \quad (11)$$

where:

$$a_6 = 2C_i^{2+}; \quad a_5 = C_i^+; \quad a_4 = 0;$$
$$a_3 = -C_i^-; \quad a_2 = -2C_i^{2-}; \quad a_1 = -3C_i^{3-};$$
$$a_0 = -4C_i^{4-}.$$

These coefficients are functions of known quantities; i.e., the total concentrations of K, Na, etc. averaged over both intra- and extramyofibrillar phases, as well as the average pH and temperature. In addition, one can estimate the relative volumes of intra- and extramyofibrillar phases from morphometric studies. These values can be incorporated into the model by expressing ion concentrations in each phase as functions of the total cellular concentrations (C_T) and V_o/V_T, the ratio of the extramyofibrillar volume to the total volume. For example, for univalent cations

$$V_o C_o^+ + V_i C_i^+ = V_T C_T^+. \quad (12)$$

Substituting $C_o^+/C_i^+ = Y$ (i.e., Eq. 3) and $s = V_o/V_T$ into Eq. 12, it can be shown that:

$$C_i^+ = C_T^+/(sY - s + 1).$$

Similarly,

$$C_i^{2+} = C_T^{2+}/(sY^2 - s + 1)$$
$$C_i^- = C_T^-/(s/Y - s + 1)$$
$$C_i^{2-} = C_T^{2-}/(s/Y^2 - s + 1)$$
$$C_i^{3-} = C_T^{3-}/(s/Y^3 - s + 1)$$
$$C_i^{4-} = C_T^{4-}/(s/Y^4 - s + 1).$$

The Donnan potential is:

$$U = (RT/F) \ln (Y). \quad (13)$$

To compute Y, insert the above expressions for the C_i's into the expressions for the coefficients $a_1 - a_6$, and then solve for Y in Eq. 11 by reiterative procedures. An initial value of Y from which the iteration proceeds can be obtained by letting $C_i = C_T$ for each ion valence. The fixed charge X^- is calculated from Eq. 2.

RESULTS AND DISCUSSION

Intact fibers

Let us compute the distribution of ions within an intact single muscle fiber. We use data for frog muscle given in Godt and Maughan (1988) and assume that the myofibrils comprise 83% of the fiber volume (Mobley and Eisenberg, 1975). Of the 17% of fiber volume outside the myofibrils, about half is occupied by longitudinal sarcoplasmic reticulum and terminal cisternae (Mobley and Eisenberg, 1975), but, for simplicity, we have assumed that all ions within the extramyofibrillar space are uniformly distributed (Somlyo et al., 1981: Table 6). We also assume that all ions within the intramyofibrillar space are uniformly distributed; thus, average concentrations of each ion are computed.

For these computations we used the values given in Table 1 appropriate to resting frog muscle at room temperature (Godt and Maughan, 1988; see their Table 1

TABLE 1 Principal ionic constituents of fluid cytoplasm of resting intact muscle fibers of the frog*

Total potassium	141
Total phosphocreatine	49.6
Total carnosine	19.5
Total magnesium	11.8
Total sodium	9
Total univalent anion‡	8.7
Total adenosine triphosphate	6.2
Total inorganic phosphate	1.4
Total parvalbumin	1
Total calcium	0.58
Average pH	7.18
Average temperature	22°C
V_o/V_T§	0.17

All concentrations expressed as mmol/l fluid cytoplasm.
*From Godt and Maughan (1988).
‡Cl^-, lactate$^-$, glycolytic intermediates and other organic anions.
§Ratio of extramyofibrillar volume to total fiber volume (Mobley and Eisenberg, 1975).

TABLE 2 Concentrations of principal ionic species of fluid cytoplasm of resting intact muscle fibers of the frog

Species	Fiber	Extramyofibrillar	Intramyofibrillar
K^+	139.99	128.19	142.41
Na^+	9.55	8.74	9.72
Mg^{2+}	1.54	1.29	1.59
$MgATP^{2-}$	5.91	7.02	5.69
ATP^{4-}	0.11	0.16	0.10
$HATP^{3-}$	0.03	0.04	0.03
$KATP^{3-}$	0.13	0.17	0.13
PCr^{2-}	46.72	55.44	44.93
MgPCr	2.88	2.88	2.88
Carn	13.03	13.03	13.03
$HCarn^+$	6.47	5.93	6.58
CaParv	0.58	0.58	0.58
MgParv	1.40	1.40	1.40
Parv	0.02	0.03	0.02
H_2P^-	0.32	0.35	0.31
HP^{2-}	0.70	0.83	0.67
MgHP	0.05	0.05	0.05
KHP^-	0.32	0.35	0.32
Other anions	8.70	9.49	8.54
Ionic strength	194	208	192

All concentrations in mmol/l fluid cytoplasm. PCr = phosphocreatine, Carn = carnosine, Parv = parvalbumin, P = inorganic phosphate, Other anions = Cl^-, lactate$^-$, glycolytic intermediates and other organic anions. Ionic strengths listed exclude myofilament charge (computed value, −42.2 meq/l). Parvalbumin binds 2 mol Ca^{2+} or Mg^{2+} per mol (see Godt and Maughan, 1988). In view of the uncertainty of its valence, parvalbumin is treated as a species with no net charge (see text). Fiber concentrations calculated from data of Table 1 and binding constants given in Godt and Maughan (1988). Extra- and intramyofibrillar concentrations calculated assuming that the extramyofibrillar volume is 0.17 that of the whole fiber (Mobley and Eisenberg, 1975). The computed concentrations of H_2ATP^{2-}, $CaATP^{2-}$, $CaHATP^{3-}$, $MgHATP^{3-}$, P^{3-}, MgH_2P^+, CaHP, CaH_2P^+, $MgCarn^{2+}$, $CaCarn^{2+}$, and CaPC are <0.01 mM each. The ADP concentration is probably <0.1 (Dawson et al., 1980).

and text). Total diffusible magnesium in the fluid cytoplasm was assumed to be 11.8 mM (Maughan and Recchia, 1985; see discussion in Godt and Maughan, 1988). Total calcium in the fluid cytoplasm was adjusted such that computed free calcium was 52 nM (Weingart and Hess, 1984). While the concentration of the calcium-binding protein parvalbumin is known (1 mM: Godt and Maughan, 1988), its valence is unknown (and would be difficult to estimate even from its structure: Tanford, 1961), so we therefore neglected the contribution of parvalbumin to the charge balance (Eqs. 1 and 2) and the calculation of ionic strength. We did, however, take into account the binding of calcium and magnesium to parvalbumin.

From our computations, we find that the total concentration of univalent anions necessary to satisfy electroneutrality within the myofibril is 50.7 meq/liter. Assuming 8.7 meq/liter are due to Cl^-, lactate$^-$, glycolytic intermediates and other organic anions (Godt and Maughan, 1988), the difference (42 meq/liter = 50.7 meq/liter − 8.7 meq/liter) is probably due to the fixed charges on the myofibrillar proteins, and is in reasonable agreement with estimates from amino acid analysis (~38 meq/liter: see Godt and Maughan, 1988, page C593). (This fixed charge estimate differs slightly from our earlier estimate of 37 meq/liter because here we use a value for total diffusible magnesium [11.8 mM] rather than a target value for free magnesium [0.8 mM] achieved by iterating total diffusible magnesium.) Computed average free magnesium in the fiber is 1.5 mM, well within the likely physiological range (Godt and Maughan, 1988). Other values are given in Table 2. Note that the calculated ionic strengths of the extra- and intramyofibrillar solutions differ by <8%,[1] justifying our assumption that for each ion the activity coefficient inside the myofibril is for all practical purposes identical to that outside the myofibril.

Table 3 summarizes the ratios of ionic concentrations between intra- and extramyofibrillar phases and the computed Donnan potential across the phase boundary. Note that the Donnan potential is small (−2.7 mV); therefore, it will contribute little to the resting membrane potential in frog muscle (~ −90 mV: e.g., Gordon and Godt, 1970).

It is also of interest to note that the average concentration of univalent anions (e.g., Cl^-) is 10% lower in the

[1]This computation excludes myofilament charge, which we provisionally assume does not contribute to ionic screening and therefore should be omitted from the ionic strength calculation.

TABLE 3 **Equilibrium distribution of ions and the donnan potential between intra- and extramyofibrillar phases of rested intact muscle fibers of the frog**

Concentration ratios ($C_{intramyofibrillar}/C_{extramyofibrillar}$)						Potential
C^+	C^{2+}	C^-	C^{2-}	C^{3-}	C^{4-}	U (mV)
1.111	1.234	0.900	0.810	0.730	0.657	−2.67

Ratios calculated from data of Table 2 and equations given in text (with $X^- = 42.2$ meq/liter and $V_o/V_T = 0.17$).

myofibrils, while that of divalent anions (e.g., PCr^{2-} and $MgATP^{2-}$) is 19% lower. Conversely, the average concentration of univalent cations (e.g., K^+) is 11% higher in the myofibrils, while that of divalent cations (e.g., Ca^{2+} and Mg^{2+}) is 23% higher. In general, from the Boltzmann relationship, for any diffusible ion with algebraic charge n:

$$C_i^n/C_o^n = (C_i^+/C_o^+)^n = (C_o^-/C_i^-)^n.$$

Therefore, a pentavalent ion, such as Fura-2, introduced into an intact muscle (at a concentration too low to affect the overall ionic balance) will have a concentration 41% lower in the myofibrillar phase than outside it. This may have some bearing on the calibration of the Ca^{2+}-induced fluorescence signals from this compound.

Skinned fibers

We can compute ion distributions and Donnan potentials in skinned or glycerinated fibers bathed in experimental solutions, assuming that the extramyofibrillar ion concentrations are fixed by those of the bath.[2] These computations can be compared with experimentally determined ion distributions and potentials. For example, Bartels and Elliott (1985) used conventional glass microelectrodes to measure potentials in skinned and glycerinated muscle from rat and rabbit. They used these potentials to compute fixed charge density using a Donnan model for a uni-univalent electrolyte. However, their solutions contained polyvalent ions (Mg, phosphate, ATP and EGTA) as well, which puts in question their computation of fixed charge density.

We used our model to compute the fixed charge density in the myofibril given their potential measurements in, for example, glycerinated rabbit muscle bathed in their standard relaxing solution (100 mM KCl, 10 mM $MgCl_2$, 20 mM phosphate, 5 mM Na_2ATP, 4 mM EGTA, pH 7). Bartels and Elliott (1985) found that the potential measured from the fibers was −2.2 to −2.3 mV which, in their calculations, yielded a computed fixed charge density of −31 meq/liter. If these potentials arise from a Donnan equilibrium,[3] our model predicts a similar fixed charge density. This agreement is not surprising since the principal salt in their solutions was KCl. A more rigorous test of our model could involve measurements in fibers bathed in solutions that better approximate the cytoplasmic fluid, i.e., where the predominant anion is PCr^{2-} (Godt and Maughan, 1988; Table 3). Our model allows us to compute such a bathing solution. The model calculations indicate that the average concentrations of ions in the myofibrillar space of an intact muscle fiber (Table 1) can be replicated by placing a skinned fiber in a bathing solution containing: 136 mM total K, 5 mM total Na, 54 mM total PCr, 12 mM total Mg, 0.58 mM total Ca, 6.8 mM total ATP, 18.6 mM carnosine, 1.5 mM phosphate, and 1 mM parvalbumin, at pH 7.18; i.e., a solution where the predominant salt is K_2PCr.

Extension of Donnan model

An apparent limitation of our model is the assumption that the fixed charges are uniformly spread throughout the myofibrillar space. Bartels and Elliott (1981; 1982; 1985) have evidence from microelectrode studies of skinned muscle fibers in ATP-containing solutions that suggests that fixed charges are uniformly distributed along the sarcomere. Other studies of this sort, however, suggest a nonuniform distribution. For example, when fibers were bathed in ATP-free solutions, Bartels and Elliott (1981; 1982; 1985) observed significantly more negative potentials in the A-band compared with the I-band. Aldoroty and colleagues (Aldoroty and April, 1982 and 1984; Aldoroty et al., 1987) measured a nonuniform distribu-

[2]In skinned muscle fibers, conventional treatment with Triton X-100 solubilizes membranous structures (e.g., the sarcoplasmic reticulum) which normally occupy part of the extramyofibrillar spaces, and the myofibrils coalesce (Aldoroty and April, 1984). This coalescence suggests that, aside from membranous structures, the extramyofibrillar space contains only diffusible material, and therefore the extramyofibrillar compartment should effectively vanish as it exchanges with the bath (an infinite compartment). Likewise, little material is left after treatment with Brij-58, because the extramyofibrillar spaces essentially vanish after osmotic compression of the skinned fiber with Dextran T-500 and do not reform after reexpansion (Umazume et al., 1986). Thus, we assume that there will be no significant potential (and concentration) gradients between the bath and extramyofibrillar compartment, and we therefore treat these two compartments as one.

[3]This is a controversial point. Godt and Baumgarten (1984) argue that the measured potential is a superposition of Donnan and diffusion potentials, the latter arising from differences in the mobilities of substrate and products of ATP hydrolysis in skinned fibers bathed in ATP-containing solutions. However, they had no means of computing the magnitude of the diffusion component. Elliott et al. (1984) argue that this diffusion potential is negligibly small compared with the Donnan potential. Experimental evidence is lacking to resolve this point, so in the present analysis we assume provisionally that the diffusion potential is indeed comparatively small and it is therefore neglected.

tion of potential in skinned crayfish muscle fibers bathed in solutions both with and without ATP. Under all conditions, they found that negative potentials were lowest in the I-band, intermediate in the A-band, and highest at the Z-line. This implies that the concentration of diffusible anions will be highest in the I-band, intermediate in the A-band, and lowest in the Z-line region (and vice versa for the diffusible cations). This is precisely the distribution of PCr^{2-} in frog muscle observed by Hill (1962). Conversely, Edelmann (1977) reports that when intact frog muscles are loaded with cesium or thallium, electron micrographs indicate that Cs^+ and Tl^+ are mainly located in the A-bands and Z-lines.

To test whether these observations can be accounted for quantitatively by the likely differences in fixed charge density along the sarcomere, we expanded our two-phase Donnan model to permit fixed charges in both phases. This was accomplished simply by including an additional charged moiety in Eq. 1:

$$C_o^+ + 2C_o^{2+} = C_o^- + 2C_o^{2-} + 3C_o^{3-} + 4C_o^{4-} + rX^-, \quad (14)$$

where r is the charge density in o as a fraction of that in i. (For simplicity, we eschewed incorporating a third phase [Z-line] into our model because the relatively high fixed charge density of the Z-line [Aldoroty and April, 1982] is likely to effectively exclude polyvalent anions.)

Hill's (1962) data are quantitative, while, unfortunately, Edelmann's (1977) are not. Thus, we considered in detail the PCr distribution in intact frog sartorius muscle shown by Hill (1962: his Fig. 1). These data indicate that about two-thirds of the total amount of PCr in the sarcomere is found within the I-band while the remaining one-third is found in the A-band. Sarcomere length was 2.61 μm; thus, given thick filaments of length 1.6 μm (Gordon et al., 1966), the total I-band length (including Z-line) is 1.01 μm. Assuming the Z-line is 0.05 μm wide, the width of the I-band (excluding Z-line) is 0.96 μm. Therefore, the ratio of I-band volume to the total volume of I-band plus A-band is 0.375 (0.96/[0.96 + 1.6]). Thus, Hill's (1962) data indicate that the concentration of PCr in the I-band is over three times ((2/0.375)/(1/0.625) = 3.33) that in the A-band. Our calculations show that if charge density in the A-band is twice that of the I-band (about that calculated by Aldoroty and April, 1984), PCr concentration in the I-band is only 11% higher than in the A-band. This obviously falls short of the concentration difference observed by Hill (1962). Even if A-band charge density is ten times higher than I-band, our calculations indicate that PCr concentration in the I-band would be only 20% higher. Thus we conclude that fixed charge differences within the sarcomere probably account for only a small fraction of the extreme concentration differences in PCr found by Hill (1962).

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