Monovalent Ions Are Spatially Bound within the Sarcomere

T. von ZGLINICKI

Department of Ultrastructural Pathology and Electron Microscopy, Institute of Pathology, Charité, Humboldt University, 1040 Berlin, German Democratic Republic

Abstract. The concentrations of sodium, potassium, chlorine, and phosphorus and the water content in A-band and I-band of rat heart muscle cells were measured by X-ray microanalysis. Concentrations per compartment water of both cations and anions are higher in the A-band, which is less hydrated than the I-band. It is concluded that at physiological ionic strength positive and negative fixed charges are effectively shielded against each other and ions of opposite sign are spatially fixed as counterions within one and the same compartment. By using sequence analysis data from the literature to evaluate the density of fixed charges in muscle it is estimated that differences in ionic concentrations between A- and I-band are chiefly due to ion binding in the A-band.

Key words: Muscle — Ions — Counterions — Ion binding

Introduction

There are intensifying discussions in the literature about the state of monovalent intracellular ions. The simplest view, found in most textbooks, of the ions as being freely dissolved in the intracellular water has been criticised in recent years by several investigators (Horowitz and Miller 1984; Edelmann 1984; Maugham and Recchia 1985; Kellermayer et al. 1986). Using independent techniques, all these authors have shown a significant degree of some kind of binding of monovalent ions to cellular polyelectrolytes. Here a further approach to that question is given by using X-ray microanalysis of frozen-dried cryosections (von Zglinicki and Bimmler 1987) to measure the distribution of ions and water between A- and I-band in rat heart muscle. Results are compared with concentrations of fixed protein charges in muscle, estimated by using sequence analysis data from the literature.

Materials and Methods

Heart muscle specimens were obtained from adult male Wistar rats by a cryobioptical technique capable of avoiding adulteration of intracellular ionic concentrations by both traumatic and ischemic injury (von Zglinicki et al. 1986). From the frozen specimens ultrathin sections about 100—200 nm thick were cut at 170—190 K in an LKB V ultrotome with cryoattachment, transferred to aluminium grids, freeze-dried at a pressure of less than 10^{-4} Torr and carbon-coated. Sections were examined at ambient temperature and 80 kV accelerating voltage in a SIEMENS Elmiskop 102 equipped with a KEVEX-7000 microanalyser. Standardization of the spectra was carried out as described elsewhere (von Zglinicki 1983). As frozen-dried sections were used, ionic concentrations per dry mass of tissue were obtained. Local water or dry mass fractions were obtained as described recently (von Zglinicki et al. 1987; von Zglinicki and Bimmler 1987). Briefly, the optical transmissions of A- and I-band were measured on micrographs from longitudinal sections (Linders et al. 1981). As established previously (von Zglinicki et al. 1987), ratios of these values are unbiased estimates of relative dry masses of the compartments. To obtain dry mass fractions in absolute terms, these values were combined with morphometrical estimates of volume densities of cellular compartments (David et al. 1981) and with estimates of the mean myocyte dry mass (von Zglinicki and Lustyik 1986). Further details of the method have been given elsewhere (von Zglinicki and Bimmler 1987).

Results

1. Experimental data

Areas probed by X-ray microanalysis and microdensitometry are in the I-band including the Z-line and in the A-band in the region where actin and myosin filaments overlap (see Fig. 1). Dry masses and concentrations of Na, K, Cl and P measured in these compartments are given in Table 1. Phosphorus measurements by X-ray microanalysis include also the P bound to nucleotides and proteins and might serve as a rough first order estimate of phosphate ion concentration only. From these results, concentration ratios according to the Donnan ratio r were calculated as indicated in Table 2. It is obvious from Table 2 that the ions do not behave like freely dissolved ions in a homogeneous Donnan system.

2. The ionic distribution model

The Debye-Huckel-length in physiological solution at room temperature is less than 0.8 nm. In other words, intracellular fixed charges separated by more than about 1 nm are effectively shielded against each other. If of opposite sign, such charges will simply compensate at low ionic strength but will attract counterions of opposite sign independently at physiological ionic strength. It seems reasonable to assume that there are a number of charges or charge clusters in and

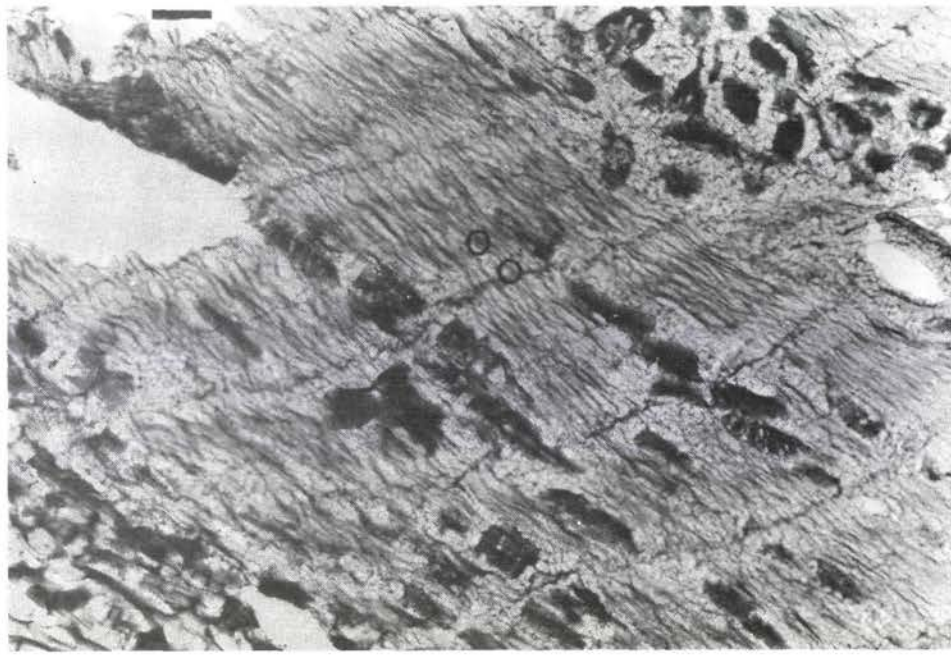

Fig. 1. Electron micrograph of a longitudinal frozen-dried cryosection of a rat heart muscle cell. Examples of the areas probed by X-ray microanalysis and microdensitometry are identified by circles. The bar corresponds to 1 μm.

Table 1. Concentrations of dry mass M_D (in % wet weight) and of elements C_X (in mmol/l of compartment water) in the A-band and I-band of rat heart muscle cells. Values are mean ± SEM. Numbers of measurements n and levels of error significance p according to the t-test (STUDENT or WELCH, respectively) are given. Measurements were performed on hearts from 5 rats.

	A-band	I-band	n	p
M_D	24 ± 1	15 ± 1	16	0.001
C_{Na}	11 ± 11	4 ± 5	20	n. s.
C_K	128 ± 13	47 ± 7	20	0.001
C_{Cl}	25 ± 3	11 ± 1	20	0.001
C_P	78 ± 10	46 ± 5	20	0.005

between biological macromolecules separated by more than about 1 nm (see below). This means that, e. g. the A-band, is not a homogeneous phase of a simple Donnan system. On the contrary, there are two interspersed phases, one with positive and one with negative fixed charges.

Table 2. Concentration ratios C_c^A/C_c^I (cations) and C_a^I/C_a^A (anions). Values are mean ± SEM.

C_{Na}^A/C_{Na}^I	C_K^A/C_K^I	C_{Cl}^I/C_{Cl}^A	C_P^I/C_P^A
2.8 ± 3.6	1.73 ± 0.24	0.44 ± 0.08	0.59 ± 0.10

To deal with it more quantitatively, a fixed cationic charge density l^+ and a fixed anionic charge density l^- (both in mval/kg of dry mass of polyelectrolytes) are defined. From the cell model of the polyelectrolyte theory (Katchalsky 1971) the concentrations of free ions of species X $C_{f,X}$ result in (Nagasawa 1974):

$$C_{f,X} \sim C_X \cdot (l \cdot M_D)^{-1} \tag{1}$$

C_X is the total concentration of ion X, l is the fixed charge density per dry mass and M_D is the dry mass concentration of polyelectrolytes. In analogy to ion pair formation free ions are defined as those separated from the fixed charge by a distance larger than the first minimum in the distribution probability.

From Eq. (1) it follows for the concentration of free cations $C_{f,c}$:

$$C_{f,c} \sim C_c \cdot (l^- \cdot M_D)^{-1} \tag{2}$$

and for the concentration of free anions $C_{f,a}$:

$$C_{f,a} \sim C_c \cdot (l^+ \cdot M_D)^{-1} \tag{3}$$

In first order only the free ions are distributed between A- and I-band according to the Donnan equation. So the Donnan equilibria for Na, K, Cl and phosphate are obtained by using Eqs. (2) and (3):

$$\frac{C_{Na}^A}{C_{Na}^I}\frac{l_I^-}{l_A^-}\frac{M_{D,I}}{M_{D,A}} = \frac{C_K^A}{C_K^I}\frac{l_I^-}{l_A^-}\frac{M_{D,I}}{M_{D,A}} = \frac{C_{Cl}^I}{C_{Cl}^A}\frac{l_A^+}{l_I^+}\frac{M_{D,A}}{M_{D,I}} = \frac{C_P^I}{C_P^A}\frac{l_A^+}{l_I^+}\frac{M_{D,A}}{M_{D,I}} = r \tag{4}$$

The subscripts A and I refer to the A- and I-band, respectively, and r is the Donnan ratio. It has been assumed that activity coefficients are identical for the free ions in both compartments. C_X^A and C_X^I are the total ionic concentrations of element X in A- resp. I-band as measured by X-ray microanalysis.

Using Eq. (4) the concentrations of cations and anions bound in, say, the A-band $C_{b,c}^A$ and $C_{b,a}^A$ may be calculated by

$$\begin{aligned} C_{b,c}^A &= C_c^A - r(C_c^I - C_{b,c}^I) \\ C_{b,a}^A &= C_a^A - 1/r(C_a^I - C_{b,a}^I) \end{aligned} \tag{5}$$

The free ions are distributed according to the remaining fixed charges not compensated by bound counterions. These remaining charges must be comparatively small; i. e., the Donnan ratio most probably will be not very different

from one (see below). Assuming this it follows from Eq. (5) that the difference in measured ionic concentrations $\Delta C_X = C_X^A - C_X^I$ is a first order estimate of the difference in bound ionic concentrations $\Delta C_{b,X} = C_{b,X}^A - C_{b,X}^I$. From the results given in Table 1 it follows that 61 ± 19 mmol/l more cations and 46 ± 12 mmol/l more anions should be bound in the A-band than in the I-band.

3. *Estimation of the fixed charge concentrations*

Fixed charge concentrations in major muscle proteins may be estimated from known sequence analysis data (Naylor et al. 1985). Starting with myosin, I refer to the data of McLachlan and Karn (1982). The myosin molecule consists of two globular heads with a net charge of 19 e^- each and a double helical tail consisting of 38 repetitive sequences, each 28 residues long. As a mean value, in each sequence one cluster of about 8 negative charges and one of about 6 positive charges exists. The repeat length is 4.2 nm, i.e., these clusters are separated by more than the Debye-Huckel-length, indeed.

With a molecular weight of 470,000 it follows for the fixed charge densities of myosin l_{my}^- and l_{my}^+:

$$l_{my}^- = 728 \text{ mval/kg dry mass and}$$
$$l_{my}^+ = 485 \text{ mval/kg dry mass.}$$

In the actin filament seven actin monomers are tightly associated with one tropomyosin and one each of the troponins. Sequence analysis data have been reported for actin by Collins and Elzinga (1975), for tropomyosin by Stone and Smillie (1978), for troponin I by Wilkinson and Grand (1975), for troponin C by Collins et al. (1977) and for troponin T by Pearlstone et al. (1977). According to these data, the total net charge per seven actin monomers is 106 e^- with altogether about 450 e^+ involved. Most of these charges will be compensated by intra- and intermolecular interactions within the actin filament. However, it might not be too optimistic to assume that about 10 % of all positive charges could be separated from the next negative charge by a distance large enough to become effectively shielded. So, one could calculate the charge densities with 156 e^- and 50 e^+ per basic unit of actin filament with a molecular weight of 517,000 to

$$l_{ac}^- = l_1^- = 302 \text{ mval/kg dry mass and}$$
$$l_{ac}^+ = l_1^+ = 97 \text{ mval/kg dry mass.}$$

In the A-band the region of complete overlap of myosin and actin filaments was measured (see Fig. 1). Here a certain fraction of fixed charges might be neutralized by hydrogen bonding to extended side chains (Bartels and Elliot 1985).

As a first order approximation this fraction might be 50 % of actin filament charges. Then, effective charge densities l_{eff} of myosin and actin filaments in the A-band are obtained:

$$l^-_{eff,ac} = 150\,mval/kg \qquad l^-_{eff,my} = 680\,mval/kg$$
$$l^+_{eff,ac} = 49\,mval/kg \qquad l^+_{eff,my} = 333\,mval/kg.$$

Fixed charge densities in the A-band l_A are the sums of the respective effective densities weighted by the dry mass concentrations of actin $M_{D,ac} = M_{D,I}$ and myosin $M_{D,my} = M_{D,A} - M_{D,I}$:

$$l^-_A = 348\,mval/kg\ dry\ mass\ and$$
$$l^+_A = 155\,mval/kg\ dry\ mass.$$

The charge densities obtained are dependent on the two above mentioned assumptions regarding the fraction of shielded charges within the actin filament and the amount of charge neutralization within the A-band. Using a second set of assumptions, namely 25 % of all positive charges in the actin filament to be shielded and no charge neutralization in the A-band, one would obtain:

$$l^-_I = 422\,mval/kg\ dry\ mass,$$
$$l^+_I = 216\,mval/kg\ dry\ mass,$$
$$l^-_A = 537 mval/kg\ dry\ mass\ and$$
$$l^+_A = 317\,mval/kg\ dry\ mass.$$

From this, the Donnan ratios r_X for ion X can be calculated by Eq. (4) (Table 3). Standard errors of these estimates are calculated only from those of ionic and dry mass concentrations, neglecting all uncertainties involved in the estimation of fixed charge densities. With these results, the assumption above of a Donnan ratio for the free ions not different from one seems to be well established. Only r_P deviates significantly from one. This is as expected because X-ray microanalysis measures total phosphorus, which can be only a very rough estimate of phosphate ions.

Concentrations of fixed charges C_F per compartment water can be calculated from the fixed charge densities per dry mass l (von Zglinicki and Bimmler 1987). These values are given in Table 4 together with the fixed charge concentration differences ΔC_F and the differences in concentrations of bound ions ΔC_b between A- and I-band calculated by Eq. (5).

Discussion

It has been shown that the ionic concentrations in heart muscle cells as measured by the X-ray microanalytical techniques used here are well in the physiological

Table 3. Calculated Donnan ratios r_X for Na, K, Cl, and P. Values are mean ± SEM. For the determination of SEM, see text. First row: Computed under the assumptions that 10% of all positive charges in the actin filament are effectively shielded and that 50% of actin filament charges and the same amount of myosin charges are neutralized in the A-band. Second row: Computed assuming 25% of positive charges in the actin filament shielded and no charge neutralization in the A-band.

r_{Na}	r_K	r_{Cl}	r_P
1.5 ± 2.4	0.94 ± 0.14	1.12 ± 0.18	1.50 ± 0.27
1.35 ± 2.2	0.85 ± 0.13	1.03 ± 0.17	1.38 ± 0.25

Table 4. Calculated fixed charge concentrations C_F (first row), differences in fixed charge concentrations ΔC_F (second row) and differences in measured concentrations of anions ΔC_a and cations ΔC_c (third row, mean ± SEM). All values are given in mmol/l compartment water. Values in brackets were computed by using the second set of assumptions (see text and legend to Table 3).

$C^+_{F,A}$	$C^+_{F,I}$	$C^-_{F,A}$	$C^-_{F,I}$
50 (102)	18 (41)	112 (173)	55 (79)
ΔC^+_F		ΔC^-_F	
32 (61)		57 (94)	
ΔC_a		ΔC_c	
46 ± 12		61 ± 19	

range (von Zglinicki et al. 1986). It has further been shown that the intracellular water and ionic distributions measured are only negligibly adulterated by freezing artifacts (von Zglinicki et al. 1987), by the special kind of preparation used and by radiation damage in the electron microscope (von Zglinicki and Uhrík 1988). The values obtained should therefore be taken as native.

Certainly, the assumptions regarding charge neutralization in the actin filament and in the A-band are quite arbitrary. However, the results given in Tables 3 and 4 show that these assumptions, including most probably also the reality case, are compatible with the main conclusion, namely that the differences in fixed charge concentrations between A- and I-band seem to be, to a large extent, compensated for by counterion binding in the A-band.

The values given in Table 4 (third row) are minimal concentrations of ions bound in the A-band, since concentrations of counterions in the second compartment cannot be estimated. However, by comparing the measured ionic concentrations (Table 1) with the computed fixed charge concentrations (Table 4, first row) it cannot be excluded, that a vast majority of the intracellular ions are spatially fixed as counterions to the polyelectrolytes.

This does not imply that counterions are bound in a chemical sense. Especially, the results presented here (Table 1) do not prove the existence of ion exchange mechanisms as assumed by the association-induction hypothesis (Ling 1977). On the contrary, although measurements of sodium concentrations by energy-dispersive X-ray microanalysis cannot be done with a high sensitivity, the results obtained for Na tend to follow the same distribution as those for K, not the opposite.

From the theoretical point of view it seems not fully clear at present how ionic activities are modified by the presence of large concentrations of fixed charges. However, decreased activity coefficients in muscle cells as compared to free ionic solutions have been repeatedly shown (Lee and Fozzard 1975; Acker et al. 1985). The mobility of counterions is only moderately decreased, and their activity will surely be larger than zero. However, the incorporation of variable activity coefficients into the derivation of Eq. (4) would not alter the qualitative conclusions drawn on the results given in Tables 3 and 4.

References

Acker H., Pietruschka F., Zierold K. (1985): Comparative measurements of potassium and chloride with ion-sensitive microelectrodes and X-ray microanalysis in cultured skeletal muscle fibres. In vitro **21**, 45—48

Bartels E. M., Elliot G. F. (1985): Donnan potentials from the A- and I-bands of glycerinated and chemically skinned muscles, relaxed and in rigor. Biophys. J. **48**, 61—76

Collins J. H., Elzinga M. (1975): The primary structure of actin from rabbit skeletal muscle. J. Biol. Chem. **250**, 5915—5920

Collins J. H., Greaser M. L., Potter J. D., Horn M. J. (1977): Determination of the amino acid sequence of troponin C from rabbit skeletal muscle. J. Biol. Chem. **252**, 6356—6362

David H., Bozner A., Meyer R., Wassiljew G. (1981): Pre- and postnatal development and ageing of the heart. Exp. Pathol. Suppl. **7**

Edelmann L. (1984): Subcellular distribution of potassium in striated muscle. Scann. Electr. Microsc. **II**, 875—888

Horowitz S. B., Miller O. S. (1984): Solvent properties of ground substance studied by cryomicrodissection and intracellular reference-phase technique. J. Cell Biol. **99**, 172s—179s

Katchalsky A. (1971): Polyelectrolytes. Pure Appl. Chem. **26**, 327—373

Kellermayer M., Ludany A., Jobst K. Szucs G., Trombitas K., Hazlewood C. F. (1986): Cocompartmentalization of proteins and K^+ within the living cell. Proc. Nat. Acad. Sci. USA **83**, 1011—1015

Lee C. O., Fozzard H. A. (1975): Activities of potassium and sodium ions in rabbit heart muscle. J. Gen. Physiol. **65**, 695—708

Linders P. W. J., Stols A. L. H., Stadhouders A. M. (1981): Quantitative electron probe X-ray microanalysis of thin objects using an independent mass determination method. Micron **12**, 1—4

Ling G. N. (1977): The physical state of water and ions in living cells and a new theory of the energyzation of biological work performance by ATP. Mol. Cell. Biochem. **15**, 159—172

Maughan D., Recchia G. (1985): Diffusible sodium, potassium, magnesium, calcium and phosphorus in frog skeletal muscle. J. Physiol. (London) **368**, 545—564

McLachlan A. D., Karn J. (1982): Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacings in muscle. Nature **299**, 226—231

Nagasawa M. (1974): Ion-binding phenomena of polyelectrolytes. In: Polyelectrolytes (Eds. E. Selegny, M. Mandel, U. P. Strauss), pp. 57—78, Dordrecht, Boston

Naylor G. R. S., Bartels E. M., Bridgman T. D., Elliot G. F. (1985): Donnan potentials in rabbit psoas muscle in rigor. Biophys. J. **48**, 47—59

Pearlstone J. R., Johnson P., Carpenter M. R., Smillie M. R. (1977): Primary structure of rabbit skeletal muscle troponin T. J. Biol. Chem. **252**, 983—989

Stone D., Smillie L. B. (1978): The amino acid sequence of rabbit skeletal a-tropomyosin. J. Biol. Chem. **253**, 1137—1148

von Zglinicki T. (1983): Quantitative Röntgenmikroanalyse biologischer Ultradünnschnitte mit Aluminium-Kohle-Aufdampfschichten als Standards. Acta Histochem. **72**, 195—201

von Zglinicki T., Lustyik G. (1986): Loss of water from heart muscle cells during aging of rats as measured by X-ray microanalysis. Arch. Gerontol. Geriatr. **5**, 283—289

von Zglinicki T., Bimmler M. (1987): The intracellular distribution of ions and water in rat liver and heart muscle. J. Microsc. **146**, 77—85

von Zglinicki T., Uhrik B. (1988): X-ray microanalysis with continuous specimen cooling: Is it necessary? J. Microsc. (in press)

von Zglinicki T., Bimmler M., Purz H. J. (1986): Fast cryofixation technique for X-ray microanalysis. J. Microsc. **141**, 79—90

von Zglinicki T., Bimmler M., Krause W. (1987): Estimation of organelle water fractions from frozen-dried cryosections. J. Microsc. **146**, 67—75

Wilkinson J. M., Grand R. J. A. (1975): Amino acid sequence of troponin I from rabbit skeletal muscle. Biochem. J. **149**, 493—496

Final version accepted March 14, 1988