Simultaneous Estimation of Intracellular Free Mg^{2+} and pH by Use of a New pH-Dependent Dissociation Constant of MgATP

Shinsuke NAKAYAMA, Hideki NOMURA, Lorraine M. SMITH, and Joseph F. CLARK*

Department of Cell Physiology, Nagoya University Graduate School of Medicine, Nagoya, 466–8550 Japan; and

* Department of Neurology, University of Cincinnati Medical Center, Cincinnati, OH 45267–0525, USA

Abstract: In the present technical note for ^{31}P -NMR, we used a new pH-dependent dissociation constant of MgATP, and re-estimated changes in the intracellular free Mg^{2+} concentration and pH

from the chemical shifts of β - and γ -ATP during Na^+ -removal in smooth muscle. We confirmed the role of Na^+ – Mg^{2+} exchange. [Japanese Journal of Physiology, 52, 323–326, 2002]

Key words: magnesium, pH, smooth muscle.

The chemical shifts of the ATP peaks in ^{31}P -nuclear magnetic resonance (^{31}P -NMR) depend on the free Mg^{2+} concentration (the ratio of free ATP to Mg^{2+} -bound ATP: $[ATP]/[MgATP]$) [1]. By the use of this Mg^{2+} dependency, the intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) has been estimated in various smooth muscle tissues. Many of these ^{31}P -NMR studies have referred to an apparent dissociation constant of MgATP (K_D^{MgATP}), which Gupta and colleagues had reported [2].

pH is known to affect the dissociation of MgATP and the intracellular pH (pH_i) in smooth muscle changes under numerous conditions [3, 4]. Some solutions, e.g., Na^+ -free solutions, used to investigate the regulation of $[Mg^{2+}]_i$ would significantly alter pH_i in smooth muscle. Bock *et al.* [5] have reported a correcting method of the apparent dissociation constant of MgATP, assuming $K_D^{MgATP} \gg K_D^{MgHATP}$. (K_D^{MgATP} and K_D^{MgHATP} are dissociation constants between Mg^{2+} and free ATP and between Mg^{2+} and HATP, respectively.) We previously reported a concomitant analysis method for $[Mg^{2+}]_i$ and pH_i , using the chemical shifts of the β - and γ -ATP peaks [6]. By applying this method to the smooth muscle of guinea pig taenia caeci, we have demonstrated that Na^+ – Mg^{2+} exchange is the main mechanism to maintain low $[Mg^{2+}]_i$ under physiological conditions. In this study we have used the correc-

tion of K_D^{MgATP} reported by Bock *et al.* [5].

McGuigan and colleagues [7] have reported new correcting formulas of K_D^{MgATP} for pH: The authors have determined the pH dependency of K_D^{MgATP} ($K_D^{MgATP}(pH)$) by directly measuring the free Mg^{2+} concentrations of model solutions, using Mg^{2+} -sensitive electrodes. In the present technical note, we incorporate the pH correction method reported by McGuigan's group into the concomitant analysis for $[Mg^{2+}]_i$ and pH_i and the re-estimate changes in these parameters upon Na^+ removal.

pH-dependency of K_D^{MgATP} described by Zhang *et al.* and Bock *et al.*

In Zhang *et al.* [7], the apparent dissociation constant of K_D^{MgATP} ($K_D^{MgATP}(pH)$) (in μM) at 25 and 37°C were expressed as quadratic functions of pH.

$$K_{D,Zhang}^{MgATP}(25^\circ C)(pH) = K_{D,Z(T_A)} = 841.793 - 142.399 \text{ pH} + 6.0 \text{ pH}^2 \quad (1A)$$

$$K_{D,Zhang}^{MgATP}(37^\circ C)(pH) = K_{D,Z(T_B)} = 1311.055 - 311.355 \text{ pH} + 19.639 \text{ pH}^2 \quad (1B)$$

Since our previous ^{31}P -NMR experiments were carried out at 32°C, we calculated $K_{D,Zhang}^{MgATP}(pH)$ at 32°C from Eqs. 1A and 1B by using the van't Hoff iso-

Received on March 6, 2002; accepted on April 12, 2002

Correspondence should be addressed to: Shinsuke Nakayama, Department of Cell Physiology, Nagoya University Graduate School of Medicine, Nagoya, 466–8550 Japan. Tel: +81–52–741–2111 (enquiry), +81–52–744–2045 (direct line), Fax: +81–52–744–2048, E-mail: h44673a@nucc.cc.nagoya-u.ac.jp

chore:

$$K_{D,Zhang}^{MgATP^{(32^\circ C)}}(pH) = K_{D,Z(T_C)} = K_{D,Z(T_B)} [K_{D,Z(T_A)}/K_{D,Z(T_B)}]^\psi \quad (1C)$$

where $\psi = (1/T_C - 1/T_B)/(1/T_A - 1/T_B)$; T_A , T_B , and T_C are absolute temperatures of 25, 37, and 32°C, respectively. For example, $K_{D,Zhang}^{MgATP^{(32^\circ C)}}(7.2 \text{ pH units})$ is calculated to be 102 μM . In the following equations, unless otherwise stated, $K_D^{MgATP}(pH)$ indicates the pH-dependency of K_D^{MgATP} at 32°C (Eq. 1C). On the other hand, Bock *et al.* [5] described the pH-dependency of the apparent dissociation constant of MgATP as follows:

$$K_{D,Bock}^{MgATP}(pH) = K_{Da}^{MgATP} \frac{1 + 10^{pK - pH}}{1 + 10^{pK - pH_a}} \quad (2)$$

According to $K_{Da}^{MgATP}(pH_a = 7.2)$ at 25 and 35°C reported by Gupta and Moore [2], at 32°C ($K_{Da}^{MgATP^{(32^\circ C)}}$) was calculated to be 41 μM using van't Hoff isochore [8]. The two pH-functions of K_D^{MgATP} (adjusted at 32°C) are plotted in Fig. 1. The ratio of $[K_{D,Zhang}^{MgATP^{(32^\circ C)}}(pH)/K_{D,Bock}^{MgATP^{(32^\circ C)}}(pH)]$ is around 2 in a physiological pH range (from 6.5 to 8.0), but it decreases significantly when pH is less than 6.5.

Simultaneous estimation of $[Mg^{2+}]_i$ and pH_i

As described above, the observed chemical shift of ATP in ^{31}P -NMR spectra depends on the ratio of free ATP to Mg^{2+} -bound ATP ($[ATP]/[MgATP]$). Since the chemical shift of β -ATP (δ_{β}) is most largely changed by Mg^{2+} -binding, this ATP peak is most frequently used to estimate $[Mg^{2+}]_i$.

$$[Mg^{2+}]_i = K_D^{MgATP}(pH) ([MgATP]/[ATP]) = K_D^{MgATP}(pH) (\delta_{\beta} - \delta_{\beta\beta}) / (\delta_{\beta\beta} - \delta_{\beta\beta}), \quad (3)$$

where $\delta_{\beta\beta}$ and δ_{β} are the chemical shifts of metal-free and Mg^{2+} -bound forms of β -ATP, respectively.

We have previously shown that the chemical shifts of metal-free and Mg^{2+} -bound β -ATP are expressed as sigmoidal functions of pH ($\delta_{\beta\beta}(pH)$; $\delta_{\beta}(pH)$) [6, 9]. Thus the estimation of $[Mg^{2+}]_i$ was corrected by intracellular pH (pH_i):

$$[Mg^{2+}]_i = K_D^{MgATP}(pH_i) (\delta_{\beta} - \delta_{\beta\beta}(pH_i)) / (\delta_{\beta\beta}(pH_i) - \delta_{\beta\beta}) \quad (4)$$

$$= F_{\beta}(\delta_{\beta}, pH_i). \quad (4')$$

The chemical shift of the γ -ATP peak has a greater pH-dependency in the biological pH range. Analogous to Eq. 4, $[Mg^{2+}]_i$ is also expressed as a function of the chemical shift of the observed γ -ATP peak (δ_{γ}) and pH_i :

Fig. 1. Graphs showing the ratio of $K_{D,Zhang}^{MgATP^{(32^\circ C)}}(pH)$ to $K_{D,Bock}^{MgATP^{(32^\circ C)}}(pH)$ (upper) and their individual changes against pH (lower) (abbreviations in text).

$$[Mg^{2+}]_i = K_D^{MgATP}(pH_i) (\delta_{\gamma} - \delta_{\gamma\gamma}(pH_i)) / (\delta_{\gamma\gamma}(pH_i) - \delta_{\gamma\gamma}) \quad (5)$$

$$= F_{\gamma}(\delta_{\gamma}, pH_i). \quad (5')$$

The substitution of the chemical shift values of the β - and γ -ATP peaks measured from an intact tissue into Eqs. 4 and 5 produces a pair of simultaneous equations of $[Mg^{2+}]_i$ and pH_i . When these two equations are solved, $[Mg^{2+}]_i$ and pH_i values are obtained concomitantly.

In our previous experiments, the chemical shifts of the β - and γ -ATP peaks were measured (relative to that of phosphocreatine) in isolated smooth muscle preparations of guinea pig taenia caeci (0.4–0.6 g) under superfusion with a “normal” solution (at 32°C, 12 ml min⁻¹) [6]. The composition of the normal solution was as follows (mM): NaCl 137.9; KHCO₃ 5.9; CaCl₂ 2.4; MgCl₂ 1.2; glucose 11.8; HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) 5; pH adjusted to 7.4–7.5 at 32°C. The sets of observed chemical shifts of β - and γ -ATP (-16.16 ± 0.03 and -2.46 ± 0.02 ppm, respectively, $n=30$) were applied to Eqs. 4 and 5, and Eq. 2 was used for a pH-function of K_D^{MgATP} . Also, pH-functions for the chemical shifts of metal-free and Mg^{2+} -bound ATP peaks (i.e., $\delta_{\beta\beta}(pH_i)$, $\delta_{\beta}(pH_i)$, $\delta_{\gamma\gamma}(pH_i)$, $\delta_{\gamma}(pH_i)$) were obtained from the measurements of model solutions [6]. The distribution of $[Mg^{2+}]_i$ was plotted against pH_i (in Fig. 3, Nakayama *et al.* [6]).

In the present study, we again estimated the distribution of $[Mg^{2+}]_i$ and pH_i (Fig. 2) by using the pH-dependency of K_D^{MgATP} reported by Zhang *et al.* [7] (Eq. 1C). (The same sets of the observed chemical shifts of

Fig. 2. Distribution of $[Mg^{2+}]_i$ and pH_i in normal solution. $K_D^{MgATP}(32^\circ C)$ (pH) and the data (sets of observed chemical shifts of β - and γ -ATP) used in Nakayama *et al.* [6] ($n=30$) were applied to this re-estimation. $[Mg^{2+}]_i$ and pH_i were concomitantly estimated by solving the simultaneous Eqs. of 4 and 5. The curves were obtained by using the means $\pm$ SD for the chemical shifts of β - and γ -peaks.

β - and γ -ATP peaks [$n=30$] were used.) $[Mg^{2+}]_i$ values are distributed from 0.61 to 1.05 mM, with an average of 0.81 ± 0.12 mM. The average value in the present estimation is about two times larger than in the previous estimation (0.33 ± 0.06 mM); however, this value is still much lower than that expected from the electrochemical gradient (1.2 mM, extracellular Mg^{2+} ; ~ -60 mV, resting membrane potential [9]). The distribution of pH_i (from 6.98 to 7.29) is the same as that of the previous simultaneous estimation: i.e., The estimation of pH_i does not depend on $K_D^{MgATP}(pH)$ because Eqs. 4 and 5 share the same $K_D^{MgATP}(pH)$. In other words, solving Eqs. 4 and 5 is essentially the same as a simultaneous estimation of pH_i and the metal-free ATP ratio, and $[Mg^{2+}]_i$ can subsequently be calculated from the metal-free ATP ratio by use of the pH-dependency of K_D^{MgATP} reported by either Bock *et al.* [5] or Zhang *et al.* [7].

Using the pH-dependency of K_D^{MgATP} reported by Zhang *et al.* [7], we also re-estimated the control values of $[Mg^{2+}]_i$ in vascular, uterus, and urinary bladder smooth muscles [10]. The values ranged from 0.7 to 0.9 mM. The pH-dependency of K_D^{MgATP} reported by Zhang *et al.* [7], and the correction using van't Hoff isochore may be useful to compare the $[Mg^{2+}]_i$ values measured with ^{31}P -NMR to those with fluorescent probes (e.g., 0.98 mM in taenia caeci [11]; 0.62 mM in cultured mesenteric artery smooth muscle [12]).

Effects of Na^+ removal and readmission

Using $K_D^{MgATP}(32^\circ C)$ (pH), we previously had also es-

Fig. 3. Changes in $[Mg^{2+}]_i$ and pH_i during the removal and readmission of extracellular Na^+ in the presence of Ca^{2+} . $[Mg^{2+}]_i$ was estimated by the use of $K_D^{MgATP}(32^\circ C)$ (pH) ($\bullet$) and $K_D^{MgATP}(32^\circ C)$ (pH) ($\blacksquare$) changes in the same manner. pH_i ($\blacktriangle$) is the same in both estimations. Extracellular Na^+ was replaced with equimolar N-methyl-D-glucamine (NMDG).

timated changes in $[Mg^{2+}]_i$ and pH_i during the removal and readmission of extracellular Na^+ [6]. The isolated smooth muscle preparation of guinea pig taenia caeci was exposed to an Na^+ -free solution substituted with equimolar N-methyl-D-glucamine (NMDG) ($n=5$). This experiment was performed in the presence of extracellular Ca^{2+} to demonstrate the physiological importance of Na^+-Mg^{2+} exchange; otherwise it might be suggested that Na^+-Ca^{2+} exchange is operated under Ca^{2+} -free conditions. In Fig. 3, a re-estimation of changes in $[Mg^{2+}]_i$ and pH_i is shown using $K_D^{MgATP}(32^\circ C)$ (pH). As in the case of the re-estimation of pH_i under control conditions (x-axis in Fig. 2), the changes in pH_i are the same in both estimations using $K_D^{MgATP}(32^\circ C)$ (pH) and $K_D^{MgATP}(32^\circ C)$ (pH). On the other hand, the use of $K_D^{MgATP}(32^\circ C)$ (pH) has provided about a twofold larger $[Mg^{2+}]_i$ ($\blacksquare$) compared to use of $K_D^{MgATP}(32^\circ C)$ (pH) ($\bullet$), but with both estimations, $[Mg^{2+}]_i$ changes in a similar manner: a slow increase in $[Mg^{2+}]_i$ during Na^+ removal and recovery of $[Mg^{2+}]_i$ after its readmission. It may be noteworthy that pH_i becomes alkaline upon the readmission of Na^+ , but $[Mg^{2+}]_i$ does not decrease below the control level. Furthermore, as shown previously (fig. 4 and table 2 in Nakayama *et al.* [6]), the intracellular ATP does not significantly change during exposure to Na^+ -

free solution (NMDG substitution). The results suggest that some mechanisms other than Mg^{2+} -buffering (including the effects of pH_i) mainly cause the $[\text{Mg}^{2+}]_i$ changes seen in Fig. 3.

Using $K_{\text{D.Bock}}^{\text{MgATP}^{(32^\circ\text{C})}}(\text{pH})$, we have previously demonstrated that the $[\text{Mg}^{2+}]_i$ increase induced by Na^+ removal is potentiated or attenuated by increasing to 12 mM, from 1.2 or decreasing the extracellular Mg^{2+} concentration (to 0 mM), respectively [6]. The same tendency of $[\text{Mg}^{2+}]_i$ changes with different extracellular Mg^{2+} concentrations is obtained by the use of $K_{\text{D.Zhang}}^{\text{MgATP}^{(32^\circ\text{C})}}(\text{pH})$, though absolute $[\text{Mg}^{2+}]_i$ values are about twofold larger at each point of ^{31}P -NMR measurement (data not shown). Further, the removal of extracellular Na^+ causes a gradual decrease in pH_i irrespective of the extracellular Mg^{2+} concentration. Therefore, the re-estimation of $[\text{Mg}^{2+}]_i$ under Na^+ -free conditions supports the proposed physiological role of Na^+ - Mg^{2+} exchange as the main mechanism to maintain low $[\text{Mg}^{2+}]_i$, even under physiological conditions [6].

The authors are grateful to Dr. H. Fukatsu and Professors T. Ishigaki and T. Tomita, Nagoya University, for their pertinent help and advice. This work was supported by research grants from Daiko Foundation (Nagoya, Japan), Goho Life Science International Fund, and Monbusho International Scientific Research Program (Japan).

REFERENCES

1. Dillon PF: ^{31}P nuclear magnetic resonance spectroscopy. In: Biochemistry of Smooth Muscle Contraction, ed. Bárány M, Academic Press, San Diego, pp 393–404, 1996
2. Gupta RK and Moore RD: ^{31}P NMR studies of intracellular free Mg^{2+} in intact frog skeletal muscle. *J Biol Chem* 255: 3987–3993, 1981
3. Aickin CC: Movement of acid equivalents across the mammalian smooth muscle cell membrane. In: Proton Passage Across Cell Membrane, Ciba Foundation Symposium 139, ed. Bock G and Marsh J, John Wiley & Sons Ltd., Chichester, pp 3–22, 1988
4. Wray S: Smooth muscle intracellular pH: measurement, regulation, and function. *Am J Physiol* 254: C213–C225, 1988
5. Bock JL, Wenz B, and Gupta RK: Changes in intracellular Mg adenosine triphosphate and ionized Mg^{2+} during blood storage: detection by ^{31}P nuclear magnetic resonance. *Blood* 65: 1526–1530, 1985
6. Nakayama S, Nomura H, and Tomita T: Intracellular-free magnesium in the smooth muscle of guinea pig taenia caeci: a concomitant analysis for magnesium and pH upon sodium removal. *J Gen Physiol* 103: 833–851, 1994
7. Zhang W, Truttmann AC, Lüthi D, and McGuigan JAS: Apparent Mg^{2+} -adenosine 5'-triphosphate dissociation constant measured with Mg^{2+} macroelectrodes under conditions pertinent ^{31}P -NMR ionized magnesium determinations. *Anal Biochem* 251: 246–250, 1997
8. Nakayama S and Tomita T: Depletion of intracellular free Mg^{2+} in Mg^{2+} - and Ca^{2+} -free solution in the taenia isolated from guinea-pig caecum. *J Physiol (Lond)* 421: 363–378, 1990
9. Nakayama S and Tomita T: Regulation of intracellular free magnesium concentration in the taenia of guinea-pig caecum. *J Physiol (Lond)* 435: 559–572, 1991
10. Nakayama S and Clark JF: Smooth muscle and NMR review: an overview of smooth muscle metabolism. *Mol Cell Biochem* (in press)
11. Tashiro M and Konishi M: Basal intracellular free Mg^{2+} concentration in smooth muscle cells of guinea-pig tenia cecum: intracellular calibration of the fluorescent indicator fraptra. *Biophys J* 73: 3358–3370, 1997
12. Touyz RM and Schiffrin EL: Activation of Na^+ - H^+ exchanger modulates angiotensin II-stimulated Na^+ -dependent Mg^{2+} transport in vascular smooth muscle cells in genetic hypertension. *Hypertension* 34: 442–449, 1999