THE EFFECT OF Mg^{++} ON THE CONFORMATION OF THE Ca^{++}-BINDING COMPONENT OF TROPONIN.

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Received September 22,1972

SUMMARY Mg^{++} like Ca^{++} induces a conformational change in the Ca^{++}-binding component of troponin. However, this change is only 36 % of the change in fluorescence intensity and 80 % of the change in optical rotation induced by Ca^{++}. The apparent binding constant of Mg^{++} to the Ca^{++}-binding component is 5×10^3 M^{-1}, much smaller than that of Ca^{++}. Circular dichroism measurements show that these changes are simple helix-coil transitions. Unlike the Ca^{++}-induced conformational change, the Mg^{++}-induced change cannot be propagated to other muscle proteins, and therefore has no physiological meaning.

INTRODUCTION

Muscle contraction is regulated by micromolar amounts of Ca^{++} [1]. Troponin (TN), a protein located on the thin filaments of muscle, is the receptor for Ca^{++} [2]. TN consists of three subunits: a component that binds Ca^{++} (TN-C), a component that inhibits the interaction between actin and myosin (TN-I), and a component that binds to tropomyosin, another protein of the thin filaments (TN-T)[3]. Recently, it has been reported that TN-C undergoes a large conformational change when the Ca^{++} concentration is changed in the micromolar range[4,5]. However, the effect of Mg^{++}, whose intracellular concentration exceeds that of Ca^{++} [6], might be considerable. Therefore, we studied the effect of Mg^{++} on TN-C, and in this communication, we present evidence that Mg^{++} can also induce a conformational change in TN-C, though not a physiologically important one.

MATERIALS AND METHODS

Preparation of Muscle Proteins. Troponin (TN) and tropomyosin (TM) were prepared from rabbit skeletal muscle by the method

of Ebashi et al[7]. TN-C and TN-T were isolated using the method of Greaser and Gergely[8]. The purity of TN-C, TN-T and TM was checked by gel electrophoresis in the presence of sodium dodecyl sulfate[9]. Fluorescence measurements showed negligible tryptophan fluorescence in TN-C and TM. The complex of TN-C and TN-T was prepared by mixing these components in 6 M urea and was dialysed against 0.3 mM $NaHCO_3$.

Fluorescence Labelling of TM. TM was mixed with the fluorescent dye, 1-dimethylaminonaphtalene 5-sulfonate (DNS), in 10 mM $NaHCO_3$ and 0.1 M KCl at $0^{o}C$. The mixture was left standing for 12 hours and was dialysed against 0.1 M KCl for two days. The molar ratio of DNS to TM in the initial mixing solution was 4:1 and was 3:1 after dialysis.

Fluorescence Measurements. A Hitachi MPF-2A fluorescence spectrophotometer was used for the fluorescence measurements. Tyrosine fluorescence of TN-C was observed at 306 nm after excitation at 276 nm. Tryptophan fluorescence of TN-T was observed at 346 nm after excitation at 290 nm in the complex of TN-C and TN-T. The fluorescence of DNS labelled to TM was observed at 500 nm after excitation at 340 nm in the complex of TN-C, TN-T and TM^{DNS}.

Optical Rotation and Circular Dichroism Measurements. A Jasco ORD/UV 5 spectrophotometer and a Jasco J-20 spectro-polarimeter were used for optical rotation and circular dichroism measurements respectively. The reduced mean residue rotation at 233 nm, $[m']_{233}$, and the mean residue ellipticity, $[\theta]$, were obtained assuming a value of 110 for the mean residue molecular weight for TN-C. Percentages of α-helix, β-structure and random-coil were calculated as described by Greenfield and Fasman[10].

Ca^{++} and Mg^{++} Concentrations. The Ca^{++} concentration was

regulated using a Ca^{++}-buffer of $CaCl_2$ and 1.6 mM EGTA (ethylene glycol bis (β-aminoethyl ether)-N,N'-tetraacetic acid) in 60 mM Na-cacodylate buffer, pH 7.3[11]. A correction was made in the calculation of the Ca^{++} concentration for a slight change in pH upon addition of $CaCl_2$. At least 1 mM EGTA was necessary to achieve the complete $-Ca^{++}$ state in TN-C at pH 7.3. We neglected the binding between Mg^{++} and EGTA because the binding constant for Mg^{++} is only 40 at pH 7.3 which is much smaller than that for Ca^{++} (4×10^7 at pH 7.3)[12]. The Mg^{++} concentration was regulated by adding $MgCl_2$ in the presence of 4 mM EGTA and 10 mM Na-cacodylate buffer, pH 6.8.

Protein Concentration. Protein concentrations were determined by the biuret method[13].

RESULTS AND DISCUSSION

Mg^{++}-induced Conformational Change in TN-C. Fig. 1 shows the effect of $MgCl_2$ on the tyrosine fluorescence intensity (I) and the optical rotation at 233 nm ($[m']_{233}$) of TN-C in the absence of Ca^{++}. Addition of 1 mM EDTA (ethylenediamine tetraacetic acid) to TN-C in the $-Ca^{++}$ state, to remove contaminating Mg^{++}, did not induce any change in the $-Ca^{++}$ state. Therefore no EDTA is necessary to achieve the $-Mg^{++}$ state.

Both I and $[m']_{233}$ increase when $MgCl_2$ is added. This means that not only Ca^{++} but also Mg^{++} can induce a conformational change in TN-C. However the change induced by Mg^{++} is only 36 % of the change in fluorescence intensity and 80 % of the change in optical rotation induced by Ca^{++}. The apparent binding constant of Mg^{++} on TN-C determined from the midpoint of the transition in Fig. 1 is 5×10^3 M^{-1}.

Change in Binding Constant of Ca^{++} to TN-C by Mg^{++}. We observed the fluorescence intensity of tyrosine in TN-C as a

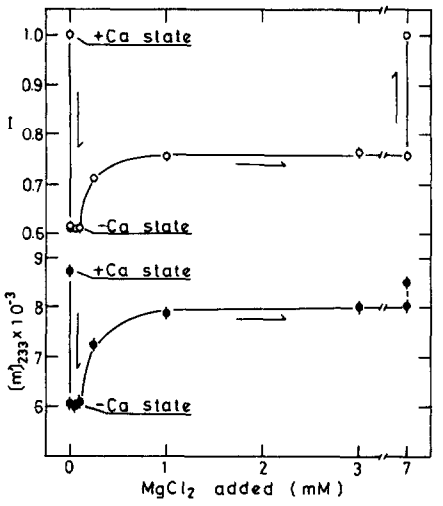

Fig. 1.

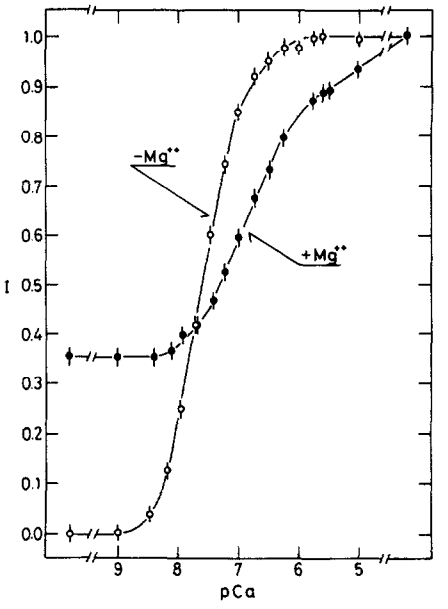

Fig. 2.

Figure 1. The effect of Mg^{++} on TN-C. o – o, relative fluorescence intensity, ● – ●, reduced mean residue optical rotation at 233 nm. Protein concentrations are 0.16 mg/ml for the fluorescence measurements and 0.2 mg/ml for the optical rotation measurements. Temperature 30°C. The initial decrease and final increase in fluorescence intensity and optical rotation are due to the addition of 4 mM EGTA and 4 mM $CaCl_2$ respectively. Vertical bars represent standard deviations.

Figure 2. The change in the fluorescence intensity of the tyrosine residues in TN-C as a function of the Ca^{++} concentration (pCa). The fluorescence intensities are expressed as a fraction of the Ca^{++}-induced change in fluorescence intensity. o – o, without $MgCl_2$, ● – ●, in the presence of 2 mM $MgCl_2$. Protein concentration is 0.09 mg/ml. Temperature 30°C. Vertical bars represent standard deviations.

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function of the Ca^{++} concentration in the presence and absence of Mg^{++} (see Fig. 2). The fluorescence intensity changes between the pCa values 8.5 and 6.0 in the absence of Mg^{++} while between 8.0 and 5.0 in the presence of Mg^{++}. The apparent binding constant of Ca^{++} to TN-C is 4×10^7 M^{-1} in the absence of Mg^{++} and 6×10^6 M^{-1} in the presence of Mg^{++}. The apparent binding constant of Ca^{++} to TN-C is shifted to a lower value

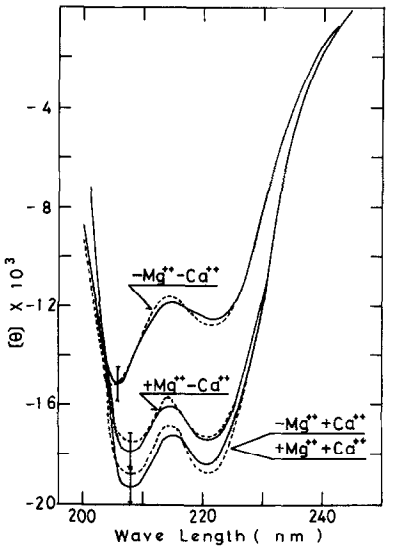

Figure 3. Circular dichroism spectra of TN-C. Continuous lines represent experimental data. Dashed lines represent reconstituted spectra calculated from the data shown in Table I. Protein concentration is 0.25 mg/ml in 10 mM Na-cacodylate buffer, pH 7.3. Temperature 27°C. The optical path length is 1 mm. 1.6 mM EGTA is present in the $-Ca^{++}$ state, no EGTA in the $+Ca^{++}$ state. 1.6 mM EGTA and 2mM $MgCl_2$ is present in the $+Mg^{++}$ state. Vertical bars represent errors due to line width.

by Mg^{++}. The binding constant of 4×10^7 M^{-1} is hihger than the one obtained before[4]. This is because, in previous experiments, we used only 0.5 mM EGTA which is too low to achieve the complete $-Ca^{++}$ state (see Method).

As shown in Fig. 2, the binding of Ca^{++} to TN-C is weakened by Mg^{++}. Also, at low pCa, the Ca^{++}-induced change in the fluorescence intensity is smaller in the presence of Mg^{++} than in the absence of Mg^{++}. These results can be understood if we assume that (1) the Mg^{++}-induced conformational change is not so large as the Ca^{++}-induced one, and that (2) Mg^{++} and Ca^{++} compete for the same binding sites.

Determination of the Secondary Structure. The secondary structure in the conformation of TN-C was calculated from circular dichroism measurements (see Fig. 3 and Table I). The

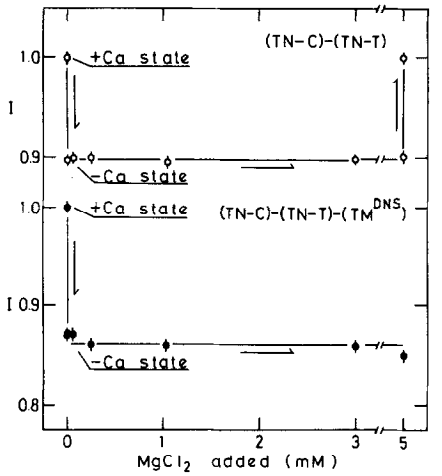

Figure 4. The effect of Mg^{++} on the complex of TN-C and TN-T, and the complex of TN-C, TN-T and TM^{DNS}. o – o, complex of TN-C and TN-T, ● – ●, complex of TN-C, TN-T and TM^{DNS}. The protein concentration is 0.23 mg/ml for the complexof TN-C and TN-T. 0.2 mg/ml TM^{DNS} and 0.1 M KCl is present in the complex of TN-C, TN-T and TM^{DNS}. Other conditions are the same as in Fig. 1. The addition of $CaCl_2$ to the complex of TN-C, TN-T and TM^{DNS} made the complex unstable and often caused precipitation to occur. Therefore, we could not observe the reversibility of the fluorescence intensity of DNS bound to TM.

Table I

Secondary structure of TN-C

	α-helix(%)	β-structure(%)	random-coil(%)
$-Ca^{++}$ $-Mg^{++}$	36	14	50
$-Ca^{++}$ $+Mg^{++}$	48	14	38
$+Ca^{++}$ $-Mg^{++}$	52	12	36
$+Ca^{++}$ $+Mg^{++}$	52	12	36

Table I. The composition of the secondary structure of TN-C calculated from the data shown in Figure 3.

results indicate that the change in conformation of TN-C are simple helix-coil transitions.

Propagation of the Conformational Change. The Ca^{++}-induced

change in TN-C can be propagated to TM via TN-T^{14}. Therefore, we examined whether the Mg^{++}-induced change could also be propagated to TN-T and to TM. TN-T contains tryptophan, which is absent in TN-C. Therefore when we observe the fluorescence of tryptophan, we can obtain information of TN-T selectively in the complex of TN-C and TN-T. Likewise, by observing the fluorescence of DNS labelled to TM, we can obtain selective information of TM in the complex of TN-C, TN-T and TM^{DNS}. Fig. 4 shows that the Mg^{++}-induced change in TN-C induces a change neither in TN-T nor in TM. Therefore, it can be concluded that the Mg^{++}-induced change in TN-C has no physiological meaning.

CONCLUSION

Not only Ca^{++} but also Mg^{++} can induce a conformational change in TN-C, but this change is not so large as the Ca^{++}-induced one. Unlike the Ca^{++}-induced change, the Mg^{++}-induced change cannot be propagated to other muscle proteins. Therefore it has no physiological meaning.

ACKNOWLEDGEMENT The authors wish to present their thanks to Prof. F. Oosawa, Prof. S. Asakura and Dr. K. Mihashi for their stimulating discussions. This work was performed while one of us JPvE held a postdoctoral fellowship of the Japan Society for the Promotion of Science.

REFERENCES

1. L. V. Heilbrunn, The Dynamics of Living Protoplasma, Acad. Press, New York, (1956).
2. S. Ebashi and M. Endo, Progr. Biophys. Mol. Biol. 18, 123, (1968).
3. S. Ebashi, T. Wakabayashi and F. Ebashi, J. Biochem. 69, 441, (1971).
4. J. P. van Eerd and Y. Kawasaki, Biochem. Biophys. Res. Comm. 47, 859, (1972).
5. A. C. Murray and C. M. Kay, Biochemistry, 11, 2622, (1972).
6. L. B. Nanninga, Biochim. Biophys. Acta, 54, 338, (1961).
7. S. Ebashi, A. Kodama and F. Ebashi, J. Biochem. 64, 465, (1968).
8. M. L. Greaser and J. Gergely, J. Biol. Chem. 246, 4226, (1971).

9. K. Weber and M. Osborn, J. Biol. Chem. 244, 4406, (1969).
10. N. Greenfield and G. D. Fasman, Biochemistry, 8, 4108, (1969).
11. G. Schwarzenbach, H. Senn and G. Anderegg, Helv. Chim. Acta, 40, 1886, (1957).
12. G. Schwarzenbach, Die komplexometrische Titration, (1955).
13. A. G. Gornall, C. J. Bardawill and M. M. David, J. Biol. Chem. 177, 751, (1949).
14. Y. Kawasaki and J. P. van Eerd, Submitted for publication.