MAGNESIUM BINDING TO CALCIUM-BINDING PROTEINS: A REGULATORY FUNCTION?

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I. INTRODUCTION

It is generally accepted that Mg$^{2+}$-ions serve a structural role in binding to the "Ca-Mg" sites of troponin C [1]. It is thus believed that Mg$^{2+}$ binding to TnC ensures that the troponin complex stays intact also in the resting, low Ca$^{2+}$, state of the muscle. The two weaker Ca$^{2+}$ sites in TnC, the regulatory sites, are normally assumed not to bind Mg$^{2+}$-ions under physiological conditions. For calmodulin there is no clear agreement regarding the Mg$^{2+}$ binding, reported values ranging from 0 to 10$^{-7}$ M$^{-1}$ [2].

We have previously used $^{43}$Ca NMR to study mainly the kinetics in the calcium binding to TnC and CaM [3]. These studies show that for both proteins there are two classes of sites with different exchange dynamics for the Ca$^{2+}$-ions. Studies of the tryptic fragment show that the C-terminal half contains the two stronger sites with slower Ca$^{2+}$-ion exchange and the N-terminal half contains the two weaker sites with faster exchange rates [4]. We have now used the same approach to study Mg$^{2+}$-ion binding to TnC and CaM by means of $^{25}$Mg NMR. These studies show undoubtedly that there are two Mg$^{2+}$ binding sites in each half of the two proteins. Furthermore, we have used a Phenyl-Sepharose column to investigate if CaM

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in which some of the Ca\(^{2+}\)-ions have been replaced by Mg\(^{2+}\)-ions, will still bind. These studies show that \((\text{Ca})_2(\text{Mg})_2\text{CaM}\) binds to the column in the same way as \((\text{Ca})_4\text{CaM}\).

II. EXPERIMENTAL

The elution experiments with Phenyl-Sepharose columns were made using a reversed salt gradient.

All NMR experiments were performed on a Nicolet 360 WB spectrometer at 22.15 MHz for \(^{25}\text{Mg}\) and 24.34 MHz for \(^{43}\text{Ca}\) using a homemade probe.

III. RESULTS AND DISCUSSION

A. Troponin C

The temperature dependence in the \(^{43}\text{Ca}\) NMR line width for a sample containing 1 mM TR\(_3\)C from TnC and an excess of Ca\(^{2+}\) is that typical for intermediate exchange and similar to TnC itself. Adding Mg\(^{2+}\)-ions results in a decrease in the \(^{43}\text{Ca}\) NMR line width. Considering reported binding constants for Ca\(^{2+}\) and Mg\(^{2+}\)-ions to TnC \([1]\) and its fragments our results are only in agreement with a model where Mg\(^{2+}\)-ions can bind to site(s) different from the Ca\(^{2+}\) binding sites and that this ion binding will modulate the dynamics in the binding of the Ca\(^{2+}\)-ions.

\(^{25}\text{Mg}\) NMR studies of Mg\(^{2+}\) binding to TnC and its tryptic fragments show that there are two strong Mg\(^{2+}\)-sites in the C-terminal half of the protein (TR\(_3\)C) with slow exchange dynamics for the Mg\(^{2+}\)-ions. In the N-terminal half of the protein (TR\(_1\)C) there are Mg\(^{2+}\) binding sites that are significantly weaker than those in TR\(_3\)C. The off-rate of the Mg\(^{2+}\)-ions from these sites are much faster (~10^3 s\(^{-1}\)) than for TR\(_3\)C. It is, however, at present not clear how many if any of these sites are the same as the Ca\(^{2+}\)-sites.

B. Calmodulin

The two stronger (slowly exchanging) Ca\(^{2+}\) sites are located in the C-terminal half of the protein whereas the two weaker (faster exchanging) sites are in the N-terminal half \([3]\). \(^{25}\text{Mg}\) NMR data show that Mg\(^{2+}\)-ions bind to both halves of CaM. It is also evident from the concentration dependence of the line width that TR\(_1\)C binds Mg\(^{2+}\)-ions stronger by a factor
of 10 than TR₂C. We have thus found that Mg²⁺-ions have a reversed site preference as compared to Ca²⁺-ions, however, the same as the lanthanides. The temperature dependence in the width of the ²⁵Mg line has been used to calculate the exchange rate of the Mg²⁺-ions resulting in an off-rate of Mg²⁺-ions from TR₁C of 2,000 - 3,000 s⁻¹, whereas the one from TR₂C is not well defined from our experiments, however, faster than for TR₁C.

The reasonably strong binding of Mg²⁺ to TR₁C and to the N-terminal half in intact CaM show that Mg²⁺ will compete favourably with Ca²⁺ for these sites, and in the non-activated cell (low calcium) these sites will be saturated by magnesium to well over 50 %, maybe 90 %. This binding can have several implications for the mechanism of CaM modulation. To test if partially Mg²⁺ loaded CaM may bind to hydrophobic sites we have studied the binding of CaM to a Phenyl-Sepharose column at different Mg²⁺ and Ca²⁺ concentrations. In the absence of Mg²⁺ a [Ca²⁺]/[CaM] ratio of 4 is needed for binding to the column. When 10 mM Mg²⁺ was present a [Ca²⁺]/[CaM] ratio of 2 was however sufficient to bind all CaM to the column. This shows that two of the four Ca²⁺-ions in CaM can be replaced by Mg²⁺-ions and the conformation of CaM still is the right one for binding to Phenyl-Sepharose and presumably to hydrophobic drugs and target enzymes. Whether or not (Ca)₂(Mg)₂CaM will also modulate the target enzyme is another question not addressed in this work.

REFERENCES


Calm

Ca²⁺-dependent family of the intesa
Ca²⁺-binds sequence of the the an atomic building proteins have been built in the skeletal The skeletal shows 46% crystal of .155. solvents This stru...