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

Torbjörn Drakenberg, Sture Forsén, Eva Thulin
and Ming-Daw Tsai[1]

Division of Physical Chemistry 2
University of Lund
Lund, Sweden

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 10^4 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,

---

[1] Permanent address: Department of Chemistry, The Ohio State University, Columbus, Ohio.

in which some of the Ca^{2+}-ions have been replaced by Mg^{2+}-ions, will still bind. These studies show that $(Ca)_2(Mg)_2CaM$ binds to the column in the same way as $(Ca)_4CaM$.

II. EXPERIMENTAL

The eluation 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}Mg and 24.34 MHz for ^{43}Ca using a homemade probe.

III. RESULTS AND DISCUSSION

A. Troponin C

The temperature dependence in the ^{43}Ca NMR line width for a sample containing 1 mM TR_1C 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}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) <u>different</u> from the Ca^{2+} binding sites and that this ion binding will modulate the dynamics in the binding of the Ca^{2+}-ions.

^{25}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_2C) with slow exchange dynamics for the Mg^{2+}-ions. In the N-terminal half of the protein (TR_1C) there are Mg^{2+} binding sites that are significantly weaker than those in TR_2C. The off-rate of the Mg^{2+}-ions from these sites are much faster ($\sim 10^3$ s^{-1}) than for TR_2C. 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}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_1C binds Mg^{2+}-ions stronger by a factor

of 10 than TR_2C. We have thus found that Mg^{2+}-ions have a reversed site preference as compared to Ca^{2+}-ions, however, the same as the lanthanides. The temperature dependence in the width of the ^{25}Mg line has been used to calculate the exchange rate of the Mg^{2+}-ions resulting in an off-rate of Mg^{2+}-ions from TR_1C of 2,000 - 3,000 s^{-1}, whereas the one from TR_2C is not well defined from our experiments, however, faster than for TR_1C.

The reasonably strong binding of Mg^{2+} to TR_1C and to the N-terminal half in intact CaM show that Mg^{2+} will compete favourably with Ca^{2+} 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 Mg^{2+} loaded CaM may bind to hydrophobic sites we have studied the binding of CaM to a Phenyl-Sepharose column at different Mg^{2+} and Ca^{2+} concentrations. In the absence of Mg^{2+} a $[Ca^{2+}]/[CaM]$ ratio of 4 is needed for binding to the column. When 10 mM Mg^{2+} was present a $[Ca^{2+}]/[CaM]$ ratio of 2 was however sufficient to bind all CaM to the column. This shows that two of the four Ca^{2+}-ions in CaM can be replaced by Mg^{2+}-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

1. Potter, J.D., Gergely, J. (1975) J. Biol. Chem. 250, 4628-4633.
2. Cox, J.A., Comte, M., Malone, A., Burger, D. and Stein, E.A. (1984) in "Metal Ions in Biological Systems", 17, 215-273.
3. Forsén, S., Drakenberg, T. and Vogel, H.J. (1986) in "Calcium and Cell Function", (Cheung, W.Y., Ed.), Vol. 6, pp. 114-157, Academic Press, New York.
4. Teleman, A., Drakenberg, T. and Forsén, S. (1986) Biochim. Biophys. Acta 873, 204-213.
5. Drakenberg, T., Forsén, S. and Lilja, H. (1983) J. Magn. Resonance 53, 4112-422.