Vol. 130, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1985 Pages 426-430

A NOTE ON Ca^{2+} BINDING TO CALMODULIN

Chih-Lueh A. Wang

Department of Muscle Research, Boston Biomedical Research Institute
20 Staniford Street, Boston, MA 02114

Received June 5, 1985

Binding of Ca^{2+} to calmodulin has been simulated on the basis of a model that assumes two classes, two sites in each class, of Ca^{2+} binding sites. With properly chosen values of binding constants for the two classes of sites, and with the assumption that certain degree of positive cooperativity exists between the two sites in each class, the overall binding isotherm can be generated so that it appears to be a single-transition, non-cooperative binding curve of four equivalent sites. Thus this model offers a resolution for some of the discrepancies among Ca^{2+} binding studies of calmodulin.

Calmodulin (CaM) is a ubiquitous protein that modulates many enzymes in eukaryotes (1-3). The regulatory action is achieved via a Ca^{2+}-dependent interaction between CaM and the target enzyme. Upon binding of Ca^{2+}, CaM undergoes a conformational change, exposing hydrophobic regions on the surface of the molecule and thereby enabling CaM to interact specifically with the enzyme (4).

CaM contains four Ca^{2+}-binding sites, numbered from I to IV from the N-terminus. Binding of Ca^{2+} can be measured either directly by equilibrium and/or flow dialysis, or indirectly by monitoring changes in various spectra -- e.g. UV absorption, circular dichroism, tyrosine fluorescence and NMR. Results on Ca^{2+} binding studies accumulated over the past decade have been rather contradictory. In most of the spectroscopic studies, for example, it was found that Ca^{2+} binding to only a fraction (usually two) of the four sites was sufficient to induce conformational changes in the CaM molecule (5-12), leading to the conclusion that there may exist two classes of Ca^{2+}-binding sites, i.e. the high and the low affinity sites. Although the presence of

Abbreviation: CaM: calmodulin.

0006-291X/85 $1.50

two-class sites is also supported by some earlier studies using equilibrium dialysis (13-15), more recent works involving direct measurements of Ca^{2+} binding (6,7,16-19) yield either only a single class of four apparently equivalent sites with little (7,16) or no (6,18) positive cooperativity, or four sites that bind Ca^{2+} in an ordered sequence (19).

The following questions are then raised: If the four Ca^{2+}-binding sites are equivalent, why would the conformational changes observed in the spectroscopic studies complete upon binding of Ca^{2+} to only two of them? On the other hand, if there are in fact two classes of site in terms of affinity, why has one failed to detect a bi-phasic transition when monitoring direct Ca^{2+}-binding? The discrepancies between the two lines of studies deserve some reconsideration.

Burger et al. (20) recently published a careful piece of work on Ca^{2+} binding of CaM by both direct (equilibrium dialysis and use of Ca^{2+} electrode) and indirect (spectroscopic) measurements. Based on their data analysis, the authors concluded that a reasonably good fit can be obtained by adopting a simple model of four identical, independent sites. The Ca^{2+}-induced structural and functional changes are interpreted as being sequential and ordered, in the sense that properties of the molecule are determined by the number of Ca^{2+} bound regardless of the sites occupied. Thus, for instance, binding of Ca^{2+} to any two of the four sites would yield the same conformation in the molecule. While this model may account for some of the spectroscopic data, it is difficult to reconcile the recent ^{43}Ca- (21,22) and ^{113}Cd-NMR data (21,23), both of which were dismissed by Burger et al.(20) on the ground of being "non-physiological". Here we would like to present an alternative point of view.

We feel that an end-point of 2 in the stoichiometric titrations monitored by spectral changes (9-12, 21,23) is a good indication of the existence of two classes, each containing two sites, of binding sites. The fact that direct measurements of bound Ca^{2+} do not produce a bi-phasic transition may be due to some other properties of metal binding, for example, cooperativity between

binding sites. To investigate this point, we have simulated binding curves based on a model that contains two independent classes of sites, two sites in each class with positive cooperativity within the class. The average number of ions bound to the 2 classes of sites of each CaM molecule is given by

$$f_i = (2K_iC + 2a_iK_i^2C^2) / (1 + 2K_iC + a_iK_i^2C^2), \quad i=1,2$$

where C is the concentration of free Ca^{2+}, K_i's and a_i's are, respectively, the intrinsic metal binding constants and interaction factors for the two classes of sites. Positive cooperativity exists when a_i's are greater than unity.

The average number of total bound ions per protein molecule is then

$$f = f_1 + f_2.$$

From a set of assumed values of K_i's, a_i's, f is calculated and plotted as a function of pCa. The simulated curve is then fitted with the Hill equation:

$$f/4 = K_aC^n/(1+K_aC^n),$$

corresponding to a single class of four binding sites with the apparent binding constant K_a and cooperativity characterized by the Hill coefficient n.

As shown in Figure 1, with a proper choice of values for K_i's and a_i's binding isotherms are generated so that, within experimental errors, the curves can be fitted with the Hill equation with n=1, mimicking a single-transition, non-cooperative binding isotherm, even though the binding curves of the individual classes of sites are well-separated.

It was pointed out by Klotz and Hunston (24) that one cannot deduce a molecular model from the fitting of binding data alone; independent information from molecular probes is necessary for relating parameters derived from binding measurements to actual binding constants. In the case of Ca^{2+} binding to CaM, although the binding data alone can be accounted for by a

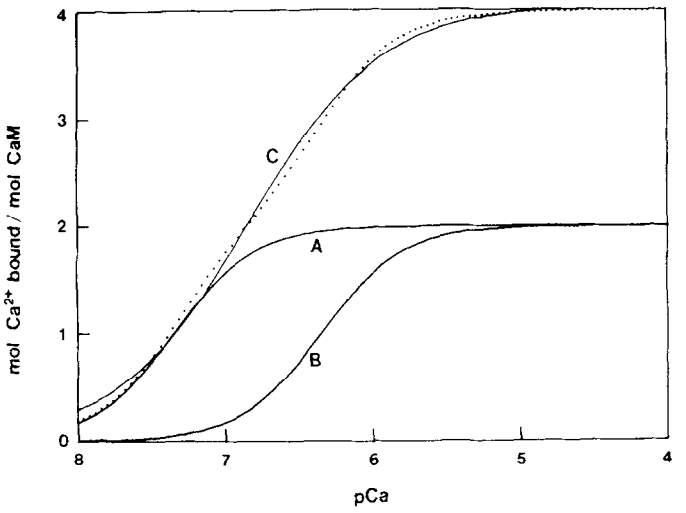

Fig. 1: Simulated binding of Ca^{2+} to CaM. Curves A and B are generated according to the equations discussed in the text with the following parameters: $K_1=5.0\times10^6$ M^{-1} and $a_1=20$ for the high affinity sites, and $K_2=5.0\times10^5$ M^{-1} and $a_2=20$ for the low affinity sites; the values of a_1 and a_2 (the interaction factors, see text) correspond to a Hill coefficient of about 1.6, indicating assumed positive cooperativity between the two sites in each class of binding sites. Dotted curve is the sum of curves A and B, representing total Ca^{2+} binding. The light line (curve C) is the best fit of the dotted curve to the Hill equation of a single class of sites; the fitted parameters are: $K_a=3.8\times10^6$ M^{-1} and n=0.97. Similar binding curves may be obtained from other sets of parameters.

relatively simple model (four equivalent sites), other experiments do suggest that more complicated schemes should be considered.

Our simulatiuon study is based on a model in which the four Ca^{2+}-binding sites of CaM fall into two classes. The binding constant of the high affinity sites (sites III and IV, according to Ref. 25) is about ten times higher than that of the low affinity sites (sites I and II); the two sites within each class interact with each other in a positively cooperative manner. The steepness of the binding curves for the two classes of sites allows good separation between them, and yet, the overall binding appears to be a single-transitioned isotherm. With this model all the experimental observations on metal binding to CaM reported to date can thus be accounted for.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Gergely for his encouragement and comments on the manuscript. This work was carried out during the tenure of a grant from NIH (AM32727).

REFERENCES

1. Cheung, W.Y. (1980) Science (Washington, D.C.) 207, 19-27
2. Klee, C.B., Crouch, T.H. & Richman, P.G. (1980) Ann. Rev. Biochem. 49 489-515
3. Klee, C.B. & Vanaman, T.C. (1982) Adv. Prot. Chem. 35, 213-321
4. LaPorte, D.C., Wierman, B.M. & Storm, D.R. (1980) Biochemistry 19 3814-3819
5. Klee, C.B. (1977) Biochemistry 16, 1017-1024
6. Dedman, J.R., Potter, J.D., Jackson, R.L., Johnson, J.D. & Means, A.R. (1977) J. Biol. Chem. 252, 8415-8422
7. Crouch, T.H. & Klee, C.B. (1980) Biochemistry 19, 3692-3698
8. Kilhoffer, M.-C., Demaille, J.G., & Gerard, D. (1981) Biochemistry 20, 4407-4414
9. Wang, C.-L.A., Aquaron, R.R., Leavis, P.C. & Gergely, J. (1982) Eur. J. Biochem. 124, 7-21
10. Ikura, M., Hiraoki, T., Hikichi, K., Mikuni, T., Yazawa, M. & Yagi, K. (1983) Biochemistry 22, 2568-2572
11. Yazawa, M., Kawamura, E., Minowa, O., Yagi, K., Ikura, M. & Hikichi, K. (1984) J. Biochem. (Tokyo) 95, 443-446
12. Klevit, R.E., Dalgarno, D.C., Levine, B.A. & Williams, R.J.P. (1984) Eur. J. Biochem. 139, 109-114
13. Lin, Y.M., Liu, Y.P. & Cheung, W.Y. (1974) J. Biol. Chem. 249, 4943-4954
14. Watterson, D.M., Harrelson, W.G., Jr., Keller, P.M., Sharief, F. & Vanaman, T.C. (1976) J. Biol. Chem. 251, 4501-4513
15. Wolff, D.J., Poirier, P.G., Brostrom, C.O. & Brostrom, M.A. (1977) J. Biol. Chem. 252, 4108-4117
16. Keller, C.H., Olwin, B.B., LaPorte, D.C. & Storm, D.R. (1982) Biochemistry 21, 156-162
17. Ogawa, Y. & Tanokura, M. (1984) J. Biochem. (Tokyo) 95, 19-28
18. Potter, J.D., Strang-Brown, P., Walker, P.L. & Iida, S. (1983) in "Methods in Enzymology" (Means, A.R. & O'Malley, B.W., eds.) Vol. 102, pp135-143.
19. Haiech, J., Klee, C.B. & Demaille, J.G. (1981) Bicohemistry 20, 3890-3897
20. Burger, D., Cox, J.A., Comte, M. & Stein, E.A. (1984) Biochemistry 23 1966-1971
21. Andersson, T., Drakenberg, T., Forsen, S. & Thulin, E. (1982) Eur. J. Biochem. 126, 501-505
22. Andersson, T., Drakenberg, T., Forsen, S., Thulin, E. & Sward, M. (1982) J. Amer. Chem. Soc. 104, 576-580
23. Andersson, T., Forsen, S., Thulin, E. & Vogel, H.J. (1983) Biochemistry 22, 2309-2313
24. Klotz, I.M. & Hunston, D.L. (1984) J. Biol. Chem. 259, 10060-10062
25. Wang, C.-L. A., Leavis, P.C. & Gergely, J. (1984) Biochemistry, 23, 6410-6415