Vol. 165, No. 2, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 15, 1989 Pages 838-844

ZINC FORMS COMPLEXES WITH HIGHER KINETICAL STABILITY THAN CALCIUM, 5-F-BAPTA AS A GOOD EXAMPLE

Péter Csermely, Péter Sándor*, Lajos Radics*
and János Somogyi

Institute of Biochemistry I., Semmelweis University
School of Medicine, Budapest, PO Box 260, H-1444 Hungary

*Central Research Institute of Chemistry,
Budapest, PO Box 17, H-1525 Hungary

Received October 30, 1989

Summary: Increasing interest is focused on the role of zinc in biological systems. A rapidly growing family of DNA-binding proteins contains "zinc-fingers", where zinc is bound to cysteine or histidine residues. On the other hand zinc is able to displace calcium from its binding sites and in this way it may modify calcium-mediated cellular processes. In the present report dissociation rates of Zn^{2+}- and Ca^{2+}-complexes with 5-F-BAPTA, a widely used NMR-active calcium indicator, have been measured by two-dimensional ^{19}F NMR exchange spectroscopic methods. The results show that the lifetime of the Zn^{2+}-complex is more than five times longer than that of the Ca^{2+}-complex. The longer lifetime, when combined with a higher thermodynamical stability of the Zn^{2+}-complex, may explain why, in some cellular processes, Zn^{2+} can compete with Ca^{2+} in spite of a presumably high $[Ca^{2+}]/[Zn^{2+}]$ free ion concentration ratio.

Calcium is well-known both as a second messenger and as a co-factor of several enzymes and cellular processes. Although zinc is generally considered as a "trace element" of moderate biological importance, in reality, it has been shown to participate in a number of cellular processes, like replication, trancription, translation, intermediate metabolism, etc. (1). In addition, many regulatory proteins and hormone receptors contain "zinc-fingers", i.e. zinc-stabilized DNA-binding structures (2). Recent reports indicate, that protein kinase C has also zinc-fingers in its regulatory domain (3,4). Our earlier findings indicate that zinc can activate protein kinase C, increases its affinity towards phorbol esters and contributes to its binding to the plasma membrane and DNA (5-7 and P. Csermely, unpublished observations).

The interrelationship between Ca^{2+}- and Zn^{2+}-mediated processes is based on the similarity of the two ions. Zn^{2+} can substitute calcium ions in Ca^{2+}-binding

0006-291X/89 $1.50

Table I.
Comparision of the affinities of calcium- and zinc-binding sites in biologically active molecules

molecule	pK_{Ca}[a]	pK_{Zn}[a]	K_{Ca}/K_{Zn}[a]	competition[b]	reference(s)
actin	5		0.2-0.7	+	31,32
	3.7	4.2	4	+	33
Ca-binding protein -- brain 21 kD	5-5.3	5.7-6	2-10		34
-- sarcoplasmic reticulum	2.8	5.1	200	+	35
Ca-channel of excitable membranes	1.7-2	2.7	5-10	+	11,12
complement C_1	4.9	4.9	1	+	36
DNA	3.9	4.4	4	+	37
mitochondrial Ca--transporter	4.6		2	+	38
myosin	3.4		0.05	+	39
phosphoinositide analogue	3.7	6.3	400		40
phospholipase A_2	4.3	6	20		41
sarcoplasmic reticulum (Ca-ATPase)	4.7	5.2	4	+	42,43
tubulin	2.3	4.3	100	+	44
yeast inorganic pyrophosphatase	2.6-3.2	5	80-400	+	45,46

[a]"K" denotes dissociation constants of both calcium- and zinc-complexes.
[b]In this column "+" means that the reference contains evidence for a direct competition of calcium and zinc. (Those quotations where both calcium and zinc affinities were given but there is evidence that their binding occurs at different sites were not included in this table.) However, we must note that the competition of calcium and zinc not necessarily mean that the two ions have common binding sites as it is shown by the example of α-lactalbumin where the competition may be a result of mutual allosteric interactions (47).

sites (Table I.). With a number of macromolecules (see Table I.), this is the consequence of a higher binding affinity of Zn^{2+} ions as compared to that of Ca^{2+}. The difference in binding affinities of the two ions, however, seldom exceeds a factor of 100. The intracellular free concentration of Zn^{2+} may be 10--1000 times lower than that of Ca^{2+} (8). This raises the question as to whether substitution of Ca^{2+} by Zn^{2+} can occur under physiological conditions. To answer this question not only thermodynamic, but kinetic studies are also needed since marked differences in the average lifetime ("kinetic stability") of Ca^{2+}-, and Zn^{2+}-complexes may substantially influence the occupation of Ca^{2+}-binding sites by these two cations.

To the best of our knowledge, no experimental data are available as to the relative lifetimes of Ca^{2+}- and Zn^{2+}-complexes with macromolecules. The direct evaluation of these kinetical parameters is rather difficult. We have therefore set up a dynamic nuclear magnetic resonance (DNMR) study of a model system where Ca^{2+} and Zn^{2+} ions are complexed with 5-F-BAPTA, a highly sensitive NMR active chelator (9). The results outlined in the sequel show that the average

lifetime of the Zn^{2+}-complex exceeds that of the Ca^{2+}-complex by a factor of 5-6.

MATERIALS AND METHODS

5-F-BAPTA (5-fluoro-1,2-bis(ortho-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid) was obtained from Molecular Probes (Eugene, Oregon, USA). $CaCl_2$ and $ZnCl_2$ were ultrapure Merck products. All the other chemicals used were of best analytical purity.

The ^{19}F NMR spectra were measured at 20 ^{o}C using a Varian XL-400 instrument operating at 376 MHz. The samples contained 20 mM histidine (pH 7.4), 8 mM 5-F-BAPTA, 1.3 mM $ZnCl_2$, 2 mM $CaCl_2$ and 20 % D_2O. The chemical shifts were referenced to external 6-fluorotryptophane (Sigma). DNMR measurements were conveniently performed by means of two-dimensional (2D) exchange spectroscopic methods. The States-Haberkorn-Ruben technique (10), was used to obtain the phase sensitive 2D chemical exchange spectra. The relevant acquisition parameters were: relaxation delay 1 sec; spectral width 3500 Hz; number of data points in t_2 512; number of t_1 increments 64, and number of transients 16. The mixing period (τ_{mix}) in successive experiments was varied between 0.5 and 50 msec. The 2D data matrices were zero filled to 512 data points in t_1 and Fourier transformed without subsequent symmetrization. Standard Varian software was used to measure the volume integrals.

RESULTS

Figure 1. displays the 2D ^{19}F chemical exchange spectrum of free 5-F-BAPTA and its Ca^{2+}- and Zn^{2+}-complexes at a mixing period (τ_{mix}) 5 msec. The diagonal peaks, labelled as "free-free", "Zn-Zn" and "Ca-Ca" represent, respectively the amounts of the free chelator and its metal-complexes that remain unchanged during the mixing period. The off-diagonal peaks, "free-Ca", "free-Zn", "Zn-free" and "Ca-free", on the other hand, correspond to the portion of the indicator which was in a certain form at the begining of τ_{mix} and, as a result of the chemical exchange process, changed to another by the end of the mixing period. The relative intensities of the "free-Ca" and "free-Zn" peaks in Figure 1. clearly indicates that the exchange rate of 5-F-BAPTA with Ca^{2+} is substantially faster than that with Zn^{2+} ions.

By repeating the 2D experiment with different τ_{mix} values, the decomposition rate of the Ca^{2+}- and Zn^{2+}-complexes can easily be obtained from the time dependence of the "Ca-Ca" and "Zn-Zn" diagonal peak intensities. The logarithmic plot of the pertinent volume integrals versus the mixing time is lienar until the overall rate of the consecutive exchange processes (i.e. the decomposition and the recombination of the metal complexes) is slow (see Fig. 2.). The slope of the linear correlation is the sum of the decomposition and the longitudinal relaxation rate constants. This latter was independently measured in inversion recovery experiments (R_1 = 1.29 $\pm$ 0.04 sec^{-1}). Taking R_1 into account, the following decomposition rate constants were calculated from the data in Fig. 2.:

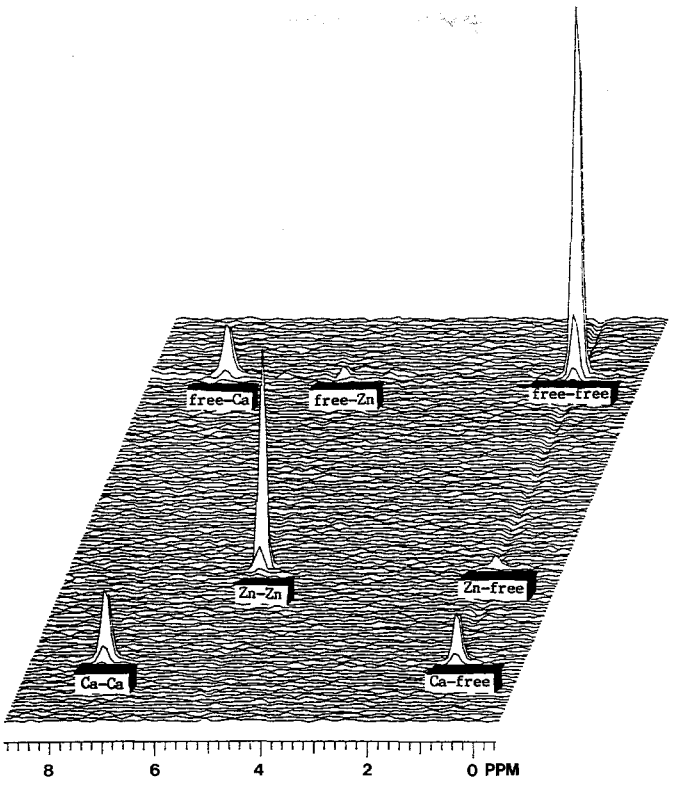

Figure 1. Two dimensional exchange spectrum of 5-F-BAPTA and its calcium- and zinc-complexes. Spectrum was recorded as it is described in Materials and Methods. For explanation of abbreviations, see text.

$k_{dec}(Zn)$ = 18.0 ± 0.5 and $k_{dec}(Ca)$ = 103 ± 4 sec^{-1}. The half lives of the Zn^{2+}- and Ca^{2+}-complexes are 6.7 and 38.5 msec, respectively.

DISCUSSION

Our data indicate that the Zn^{2+}-complex of 5-F-BAPTA has a 5-6-times longer half life than the Ca^{2+}-complex of the chelator. This kinetic stability of the Zn^{2+}-5-F-BAPTA is in agreement with the data of Hagiwara and Kawa (11,12) who

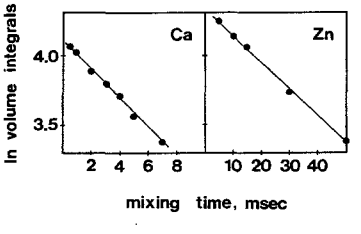

Figure 2. Logarithmic plot of the "Ca-Ca" (panel "Ca") and "Zn-Zn" (panel "Zn") volume integrals versus the mixing time as obtained from phase sensitive 2D exchange spectra. For details, see text.

demonstrated that zinc moves 10-20 times slower through the calcium channels of excitable membranes than does calcium itself.

To judge the physiological relevance of our data one has to compare the structure of macromolecular Ca^{2+}- and Zn^{2+}-binding sites with the structure of BAPTA--complexes. Our model compound, 5-F-BAPTA chelates Ca^{2+} and Zn^{2+} forming an octahedral structure of four carboxyl groups, two ether oxygen atoms, and two anilinic nitrogens (13). In most of the Ca^{2+}-binding sites Ca^{2+} is chelated also in an octahedral structure of carboxyl and hydroxyl groups or carbonyl oxygens of the peptide bond (14). Carboxyl groups also play a significant role in Zn^{2+}--binding sites together with the imidazole nitrogens of histidine side-chains (15). These structural similarities support the hypothesis that the Zn^{2+}-complexes of macromolecules may also have a longer half life (higher kinetical stability) than the corresponding Ca^{2+}-complexes. To test the validity of this hypothesis, however, further studies are needed.

There are several examples which demonstrate that relatively low concentartions of zinc can antagonize the effect of calcium. Zinc is able to inhibit the calcium transport of mitochondria (16,17), platelets (18), and red blood cells (19). Zinc antagonises the calcium-dependent smooth muscle contraction (20) and neurotransmitter release (21). The review of Habermann and Richardt (22) summarizes a number of cases where calcium is substituted by other heavy metals.

These examples give further support to the assumption, that zinc can actually displace calcium from many of its binding sites even at much lower free concentration than that of calcium. Zn^{2+} may form a kinetically stable complex preventing calcium to reassociate with its original binding site. In this way zinc has the possibility to modify calcium signals in living cells. However, we would like to point out that the calcium-zinc antagonism is not the only way of the interaction of these two cations. Zinc and calcium have also similar effects in the excitation of neurones (11) and in membrane stabilization (23). Zinc not only blocks but also induces calcium fluxes (e.g.: in red blood cells (24), hepatocytes (25) and sarcoplasmic reticulum (26)) or has no siginificant effect on calcium signals (27). This variability of the action of zinc can be explained by the great variety of its binding sites demonstrated in Table I. In this way different effects of zinc can be the most characteristics in different zinc concentrations.

The greater thermodynamical and kinetical stability of some zinc complexes may provide an explanation to our earlier finding that the activation of protein kinase C impairs the calcium transport of the calcium-ionophores A23187 and ionomycin (28). Prolonged activation of protein kinase C induces the translocation of zinc from the nucleus and mitochondria to the plasma membrane and cytosol (29,30). The increased amount of zinc in the cytosol may form thermodynamically and kinetically more stable complexes with the calcium-ionophores than calcium itself thus prevent-

ing calcium to enter the cells via these phores. This explanation is further supported by the finding that TPEN, an intracellular heavy metal chelator restores the ionophore-induced calcium transport (28) possibly by chelating the former "zinc--cork". This mechanism may operate in the regulation of calcium channels and certain calcium binding proteins as well.

ACKNOWLEDGMENTS

This work was supported by research grants from the Hungarian National Academy of Sciences and the Ministry of Social Welfare and Health.

REFERENCES

1. Vallee,B.L. (1986) In: Zinc Enzymes (I.Bertini, C.Luchinat, W.Maret and M. Zeppezauer, Eds.), Vol. I. pp. 2-15. Birkhauser, Boston.
2. Berg,J.M. (1986) Science 232, 485-486.
3. Parker,P.J., Coussens,L., Totty,N., Rhee,L., Young,S., Chen,E., Stabel,S., Waterfield,M.D. and Ullrich,A. (1986) Science 233, 853-859.
4. Rosenthal,A., Rhee,L., Yadegari,R., Paro,R., Ullrich,A. and Goeddel,D.V. (1987) EMBO J. 6, 433-441.
5. Csermely,P., Szamel,M., Resch,K. and Somogyi,J. (1988) J. Biol. Chem. 263, 6487-6490.
6. Csermely,P., Szamel,M., Resch,K. and Somogyi,J. (1988) Biochem. Biophys. Res. Commun. 154, 578-583.
7. Csermely,P. and Somogyi,J. (1989) Proc. of the 17th International Conference on Cyclic Nucleotides and Protein Phosphorylation, Kobe (Japan), pp. 128.
8. Metcalfe,J.C., Hesketh,R.T. and Smith,G.A. (1985) Cell Calcium 6, 183-195.
9. Smith,G.A., Hesketh,R.T., Metcalfe,J.C., Feeney,J. and Morris,P.G. (1983) Proc. Natn. Acad. Sci. USA 80, 7178-7182.
10. States,D.J., Haberkorn,R.A. and Ruben,D.J. (1982) J. Magn. Reson. 48, 286-292.
11. Hagiwara,S. and Byerly,L. (1981) Ann. Rev. Neurosci. 4, 69-125.
12. Kawa,K. (1979) J. Membr. Biol. 49, 325-344.
13. Gerig,J.T., Singh,P., Levy,L.A. and London,R.E. (1987) J. Inorg. Biochem. 31, 113-121.
14. Kretsinger,R.H. (1976) Ann. Rev. Biochem. 45, 239-266.
15. Christianson,D.W. and Alexander,R.S. (1989) J. Am. Chem. Soc. 111, 6412-6419.
16. Brierley,G.P. (1967) J. Biol. Chem. 242, 1115-1122.
17. Batra,S. (1973) J. Cell. Physiol. 82, 245-256.
18. White,G.C.II. and Raynor,S.T. (1982) Biochem. Biophys. Res. Commun. 104, 1066--1072.
19. Varecka,L., Peterajova,E. and Pogady,J. (1986) Biochim. Biophys. Acta 856, 585-594.
20. Sarria,B., Cortijo,J., Marti-Cabrera,M., Morcillo,E. and Esplugues,J. (1989) Br. J. Pharmacol. 97, 19-26.
21. Nishimura,M. (1987) Pflugers Arch. 410, 623-626.
22. Habermann,E. and Richardt,G. (1987) Trends Pharmacol. Sci. 7, 298-300.
23. Bashford,C.L., Alder,G.M., Patel,K. and Pasternak,C.A. (1986) J. Biol. Chem. 261, 9300-9308.
24. Plishker,G.A. (1984) Am. J. Physiol. 247, C143-C149.
25. Parker,J.C. and Barritt,G.J. (1981) Biochem. J. 200, 109-114.
26. Carvalho,A.P. (1972) Eur. J. Biochem. 27, 491-502.
27. Beaven,M.A., Rogers,J., Moore,J.P., Hesketh,T.R., Smith,G.A. and Metcalfe,J.C. (1984) J. Biol. Chem. 259, 7129-7136.
28. Csermely,P. and Somogyi,J. (1989) J. Cell. Physiol. 138, 593-602.
29. Csermely,P., Gueth,S. and Somogyi,J. (1987) Biochem. Biophys. Res. Commun. 144, 863-868.

30. Csermely,P., Fodor,P. and Somogyi,J. (1987) Carcinogenesis 8, 1663-1666.
31. Kasai,M. and Oosawa,F. (1968) Biochim. Biophys. Acta 154, 520-528.
32. Strzelecka-Golaszewska,H. (1973) Biochim. Biophys. Acta 310, 60-69.
33. Strzelecka-Golaszewska,H., Prochniewicz,E. and Drabikowski,W. (1978) Eur. J. Biochem. 88, 229-237.
34. McDonald,J.R., Walsh,M.P., McCubbin,W.D., Oikawa,K. and Kay,C.M. (1985) Biochem. J. 232, 569-575.
35. Ikemoto,N., Nagy,B., Bhatnagar,G.M. and Gergely,J. (1974) J. Biol. Chem. 249, 2357-2365.
36. Ziccardi,R.J. (1983) J. Biol. Chem. 258, 6187-6192.
37. Waalkes,M.P. and Poisier,L.A. (1984) Toxicol. and Appl. Pharmacol. 75, 539-546.
38. Jeng,A.Y., Ryan,T.E. and Shamoo,A.E. (1978) Proc. Natn. Acad. Sci. USA 75, 539-546.
39. Beinfeld,M.C., Bryce,D.A., Kochavy,D. and Martonosi,A.N. (1975) J. Biol. Chem. 250, 6282-6287.
40. Hendrickson,H.S. and Reinertsen,J.L. (1969) Biochemistry 8, 4855-4858.
41. Wells,M.A. (1973) Biochemistry 12, 1080-1085.
42. Carvalho,A.P. (1966) J. Cell. Physiol. 67, 73-84.
43. Wakabayashi,S. and Shigekawa,M. (1987) J. Biol. Chem. 262, 11524-11531.
44. Jemiolo,D.K. and Grisham,C.M. (1982) J. Biol. Chem. 257, 8148-8152.
45. Ridlington,J.W. and Butler,L.G. (1972) J. Biol. Chem. 247, 7303-7307.
46. Baykov,A.A. and Avaeva,S.M. (1974) Eur. J. Biochem. 47, 57-66.
47. Murakami,K. and Berliner,L.J. (1983) Biochemistry 22, 3370-3374.