Laboratori Nazionali di Frascati

LNF-87/38

A. Giovannelli, I. Ascone, A. Bianconi, A. Congiu, S. Alemà and P. Fasella:

CALCIUM BINDING SPECIFICITY AND LOCAL STRUCTURE IN CALCIUM MODULATED PROTEINS BY HIGH RESOLUTION XANES AND EXAFS SPECTROSCOPY

Estratto da:
SIF - Conf. Proc. "Synchrotron Radiation at Frascati" Vol. 5, 71 (1986)

Servizio Documentazione
dei Laboratori Nazionali di Frascati
P.O. Box, 13 - 00044 Frascati (Italy)

CALCIUM BINDING SPECIFICITY AND LOCAL STRUCTURE IN CALCIUM MODULATED PROTEINS BY HIGH RESOLUTION XANES AND EXAFS SPECTROSCOPY

A.Giovannelli, I.Ascone, A.Bianconi, A.Congiu
Dip. di Fisica, Universitã "La Sapienza" di Roma, Roma, Italy

S.Alemà
Istituto di Biologia Cellulare del CNR, Roma, Italy

P.Fasella
Comm. European Community, Bruxelles, Belgium

Calcium has been established to have the fundamental messanger role in the regulation of cellular processes[1]. This is carried on by the interaction of Ca^{2+} with a class of calcium modulated proteins which bind and relase Ca^{2+} as a function of its intracellular concentration ranging from 10^{-7} M (resting state) to 10^{-5} M (excited state).

Here we report the results of an extensive X-ray spectroscopy investigation on the local structure at the Ca^{2+} of four Ca modulated proteins (CaMP): calmodulin (CaM), an ubiquitous and multifunctional regulator of ATPase activity, troponin C (TnC), a skeletal muscle specific CaMP, carp parvalbumin (CPa), essentially located in carp white muscle but also in mammalian cells and S100, a dimeric brain specific CaMP whose function is still unclear. CaM and TnC bind four Ca ions per protein, CPa two and S100 one per monomer. All these proteins have been purified taking care of avoiding Ca contamination. Tests have been performed to verify their purity, functional activity and Ca content. Samples have been prepared as previously described[2]. XANES and EXAFS spectra have been measured at the Frascati synchrotron radiation facility PULS, with the storage ring operating at 1.5 GeV and 60-30 mA, by steps of 0.2 eV and 1.0 eV respectively.

We have found that the EXAFS spectra give the same first shell (Ca-O) mean distance (2.41 0.01 A) for $CaMCa_2$ and $CPaCa_2$. This is in agreement with the large similarity found in the XANES spectra of the two proteins, which differ only in little details, probably due to small distortions of the COO^- groups coordinating the Ca ion in the binding sites. In fact, theoretical XANES calculations for a cluster formed by a Ca ion coordinated by 8 COO^- groups evidence the features of the XANES peaks as depending on the COO^- geometry[2]. No appreciable differences have been found in the spectra of $CaMCa_4$ (both high, 10^{-6} M, and low, 10^{-4} M, affinity

sites occupied) respect to those of $CaMCa_2$ (high affinity). Despite the high resolution crystal structure of CPa is known, these results are not sufficient to deduce that the two local structures are the same.

The comparison between the spectra of CPa, CaM and S100 (in the dimer state), all containing two Ca ions per molecule, shows differences between S100 and the first two both in the position and in the shape of the XANES features, that appear closer to these of whiting CPa, a mutant CPa binding only one Ca ion in an high affinity site. The energy separation between the edge (ΔE) and the first multiple scattering resonance is however the same, indicating a similar first coordination shell distance[3]. This suggests that while the general features of the site are conserved, the conformation of the site is different from those previously observed.

A quite different site conformation has been found analizing the spectra of $TnCCa_2$, where the energy separation ΔE is shifted of -0.8 eV and this shift is propagated over the whole spectrum. The mean first shell distance is longer than in the other CaMP, and no difference is appreciable comparing the spectra with two and four Ca ions bond. Unlike observed on CaM, CPa and S100 the presence of Mg (necessary for the functional activity) induces a reduction of the energy separation ΔE.

In conclusion we find that the similar features present in the XANES spectra of all the CaMP studied confirm their common origin from a single site ancestor. During the evolution extremely specialized CaMP species, like S100 and TnC appeared, the last characterized by a particular Ca binding site geometry.

Although at the moment it's not possible to define a precise relation between Ca affinity, specificity and local structure, we observe different geometries in CaMP sites whose affinity is about 10^{-7} M (CaM III and IV, CPa, S100) and in the sites III and IV of TnC, whose affinity is about 10^{-8} M and bind both Ca and Mg ions. These observations suggest that different local structures can be necessary to achieve higher affinity values, with some loss in specificity. Further this assertion can be corroborated by the comparison of the low resolution crystal structures of CaM[4] and TnC[5], which present different geometries regarding the linker elical region between the two high affinity sites, probably producing differences in the local structure appreciable by the XANES spectra.

References:

(1) R.H.Kretsinger, CRC Crit. Rev. Biochem. 8, 119 (1980).
(2) A.Bianconi, A.Giovannelli, L.Castellani, S.Alemã, B.Oesh and S.Mobilio, J. Mol. Biol. 165, 170 (1983).
(3) C.R.Natoli, in EXAFS and Near Edge Structures, Ed. by A.Bianconi, L.Incoccia and S.Stipcich (Springer Verlag, 1983), p. 43.
(4) B.Y.Sudhakar et al., Nature 315, 37 (1985).
(5) O.Herzberg and M.N.G.James, Nature 313, 653 (1985).