CHAPTER 4

Calcium Binding Constants Database of Calcium Binding Proteins

4.1 Description about the database

The EF-Hand Calcium-Binding Proteins Data Library (EF-Hand CaBP-DL) is a highly curated collection of sequence of the EF-Hands with calcium binding affinity of EF-Hand superfamily of calcium-binding proteins. It has been conceived, designed, and implemented with a sole aim to find a relationship among the variation in the calcium binding abilities among the different members of EF-hand superfamily across the species.

All information that is not obtained directly from another public database has been published in a peer-reviewed journal. Each data associated with the library has been checked and validated it for inclusion in the database. The data library has the reference associated with every particular piece of information which can be used for checking the methodologies used for the determination of calcium binding constants.

As can be seen in data library, the information in the database is organized around proteins. Each mutant or isoform of a protein is considered a unique protein in the database, and is treated separately. This allows storage of the calcium-binding constants, for instance, of two isoforms of parvalbumin from the same species, and clearly indicates that the two sets of binding constants are for two different chemical entities. It also allows storage of any type of information about a mutant, thereby giving a complete picture about the variation in calcium binding constants among the native and its mutant due to the mutation of EF hand loop.

All of the isoforms and mutants of a given protein associated by a common group are stored in the database. This allows correlating calcium binding constants of isoforms and mutants of a given protein from the evolution perspective.

The EF-Hand CaBP-DL is divided into three main sections: general information (name of the protein), sequence information (EF hand sequence) and Reference.

- **General Information** includes the main entry point for information about a particular protein. The General Information for a protein summarizes name of the protein and to the organism which it belongs.
- **Sequence Information** includes information about the amino acid composition of the binding loops.
- In addition to these informations, there is also a section that includes lists of references.

Data integrity is also ensured by the requirement for the inclusion of the reference from which the information was obtained. Only data that has been published in a peer-reviewed article is included into the database.

4.2 Results and Discussion

Table 4.3

(1) Calcium Binding Constants for Calcium Binding Proteins

Sequence	Ca^{2+} binding cons.	Loop	Name	Reference
DKDGDGTITT KE	1×10^7	Loop1	Bovine cam	(1991) *J.B.C.* 266, 8050-8054.
DADGNGTIDFPE	3.98×10^7	Loop2	Bovine cam	(1991) *J.B.C.* 266, 8050-8054.
DKDGNGY ISAAE	3.16×10^6	Loop3	Bovine cam	(1991) *J.B.C.* 266, 8050-8054.
DIDGDGQVNYEE	2.5×10^6	Loop4	Bovine cam	(1991) *J.B.C.* 266, 8050-8054.
DTDGSGTIDAKE	8.30×10^5	Loop1	caltractin chalmyd	(1994) *J.B.C.* 269, 15795-15802.
DKDGSGTIDFEE	8.30×10^5	Loop2	caltractin chalmyd	(1994) *J.B.C.* 269, 15795-15802.
DNSGTITI KDLR	6.25×10^3	Loop3	caltractin chalmyd	(1994) *J.B.C.* 269, 15795-15802.
DKDGSGTIDFEE	6.25×10^3	Loop4	caltractin chalmyd	(1994) *J.B.C.* 269, 15795-15802.
DKDGDGCITTRE	3.80×10^5	Loop1	human cam-like protein	(1992) *Bioch.* 31, 12826-12832
DRDGNGTVDFPE	1.90×10^5	Loop2	human cam-like protein	(1992) *Bioch.* 31, 12826-12832
DKDGNGFVSAAE	4.90×10^4	Loop3	human cam-like protein	(1992) *Bioch.* 31, 12826-12832
DTDGDGQVNYE	1.20×10^4	Loop4	human cam-like protein	(1992) *Bioch.* 31, 12826-12832
DQDKSGFIEEDE	2.7×10^9	Loop1	parvalbumin cyprinus	(1980) *Eur J Bioch.* 111, 73-78
DSDGDGKIGVDE	2.7×10^9	Loop2	parvalbumin cyprinus	(1980) *Eur J Bioch.* 111, 73-78
DTLIKRELKQLITKE	2.7×10^7	Loop1	S100A12 Sus	(1994) J.B.C.269, 28929-28936.
DANQDEQVSFKE	6.50×10^4	Loop2	S100A12 Sus	(1994) J.B.C.269, 28929-28936.
DPNQLSKEELKLLLQTE	1.6×10^8	Loop1	Calbindin D9k Bovine	(1991) *Bioch.* 30, 154-183
DKNGDGEVSFEE	4×10^8	Loop2	Calbindin D9k Bovine	(1991) *Bioch.* 30, 154-183
DKDKSGTLSVDE	$(2.9 \pm 0.3) \times 10^5$	Loop1	S100 like liver lung fish	*Eur. J. Biochem.* 269, 3433-3441 (2002)
DTNKDGQVSWQE	$(6.0 \pm 0.7) \times 10^3$	Loop2	S100 like liver lung fish	*Eur. J. Biochem.* 269, 3433-3441 (2002)
ELDTLGEESYKD	5.5×10^4	Loop1	GF14 Arabidopsis	The Plant Cell, Vol. 6, 501-510, 1994
VQFDTCHNLDA	4×10^4	Loop1	lima bean lectin	Plant Physiol. (1991) 95, 286-290
DSFDTDSKGFITPE	1.6×10^6	Loop1	Trop C alpha cray fish	264, 30, JBC , 18240-18246,1989
DGSGEIEFEEFAE	8.1×10^3	Loop2	Trop C alpha cray fish	264, 30, JBC , 18240-18246,1989
DRGGDGYITTQVLRE	1.1×10^3	Loop3	Trop C alpha cray fish	264, 30, JBC , 18240-18246,1989
DSFDTDSKGFITPE	1.9×10^4	Loop1	Trop C gamma cray fish	*Eur. J. Biochem.* 269, 3433-3441 (2002)
DGSGELEFEEFVE	8.1×10^2	Loop2	Trop C gamma cray fish	*Eur. J. Biochem.* 269, 3433-3441 (2002)
DPDKPGKILLMD	0.014±0.005μMdisCon	Loop1	Rabbit Serum Paraoxonases	Drug Met. and Disp. 26, 7, 653-660,1998
DEDNIVYLMVVN	5.31±0.94μMdissoCon	Loop2	Rabbit Serum Paraoxonases	Drug Met. and Disp. 26, 7, 653-660,1998
NPNSPGKILLMD	0.38± 0.09μMdisCons	Loop1	human Serum Paraoxonases	Drug Met. and Disp. 26, 7, 653-660,1998
DEDNAMYLLVVN	6.2±1.2μMdissConsta	Loop2	human Serum Paraoxonases	Drug Met. and Disp. 26, 7, 653-660,1998

Ca^{2+} binding cons. - Calcium binding constant

dis. Cons. – Dissociation constant

Loop – Calcium binding loop

Name - Organism name

4.2.1 Determinants of Ca^{2+} affinity

For a clear understanding of the Ca^{2+} binding ability of the various EF-hands, four factors must be considered. The first is the intrinsic Ca^{2+} affinity of each EF-hand (ΔG_{intr}), which mirrors both the ligands presence and the contributions of non coordinating interactions. The second is the ability of a given EF-hand to differentiate between Ca^{2+} and Mg^{2+}, a cation chemically similar to Ca^{2+} that can pose significant selectivity problems due to its high cytosolic concentration. The third factor is co-operativity, a phenomenon that enables an EF-hand pair to bind Ca^{2+} as a unit. The final factor for those EF-hand proteins is the presence of target proteins which also somehow affect the calcium affinity. Taken together, the observed Ca^{2+} affinity of an EF-hand can be described as:

$$\Delta G_{obs} = \Delta G_{intr} + \Delta\,\Delta G_{sel} + \Delta\,\Delta G_{co\text{-}op} + \Delta\,\Delta G_{interact} \quad (1)$$

Where the effects of Mg^{2+} selectivity ($_{sel}$), co-operativity ($_{co\text{-}op}$) and target interaction ($_{interact}$) are seen as an energetic coupling ($\Delta\,\Delta G$) that reflects the difference in affinity [ΔG_{tot} (total free-energy change) of binding] in the presence and absence of these factors (e.g. $\Delta\,\Delta G_{sel} = \Delta G_{tot\text{ , presence of Mg}} - \Delta G_{tot\text{ , absence of Mg}}$). So only one term that is independent of any other factor is ΔG_{intr} otherwise for the calculation of contribution towards calcium binding affinity by other terms in the equation (1) one has to take into account other factors.

4.2.2 Determinants of intrinsic Ca^{2+} affinity

The EF-hand presents an intriguing mystery, as the Ca^{2+} dissociation constants found in this protein family range from 10^{-9} to 10^{-4} M. If one considers thermodynamics and the following well-known equations, an understanding can be made:

$$\Delta G = -RT \cdot \ln K_a \quad (2)$$

Where R is the gas constant and T is the temperature.

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

Where ΔH is the change in the enthalpy of the system, T is the temperature in degrees kelvin and ΔS is the change in entropy. The intrinsic Ca^{2+} affinity is determined by the difference in the Gibbs' free energy (ΔG) between the unbound and bound states. Clearly, the larger and more negative the ΔG, the higher the affinity. At a constant temperature,

phenomena that increase ΔS or decrease ΔH between the unbound and bound states should lead to higher affinity.

One factor mentioned in equation (3) is the entropy and this can be analysed further in order to understand the complexities of the interaction between the calcium and EF hand loop.

4.2.3 Entropic contributions to affinity

It is a well documented phenomenon that on binding of the calcium to the EF hand loop there is a release of frozen water molecules from its coordination spheres to the bulk solvent. An event that is fairly constant per bound calcium and this lead to increase in solvent entropy (Linse et al., 1995). This increase in solvent entropy is large enough to favour the increase in ΔG. As a result of the exchange of water molecules with the bulk solvent there is an increase in calcium affinity. So greater the exchange of water molecules, greater is the calcium affinity.

The above mentioned fact can be highlighted by the identity of ninth and twelfth loop positions. The coordinating side chain of a glutamic acid or glutamine residue in the ninth position is large enough to form linkage with the bound calcium ion directly. If the coordinating side chain is small, it has to take the help of water molecules to bridge the gap (Rake et al., 1996). The consequence of this is apparent when the EF-hands of parvalbumin & subfamily member oncomodulin are taken into account. In case of EF2 of oncomodulin there is an aspartic acid residue in the ninth position and a water molecule in its co-ordination sphere instead of glutamic acid. The resulting loss in increased solvent entropy due to the more incomplete loop chelation is thought to contribute to the lower affinity of this site compared with that of EF3 of oncomodulin and those found in parvalbumin (Lee et al., 2004).

In cases where an aspartic acid residue is found instead of a glutamic acid residue in position 12 of the EF-loop the above mentioned explanation can be applied in the same manner. This can be further corroborated by the crystal structure of CIB (calcium and integrin binding protein) where in the aspartic residue has to rely upon the water

molecule to complete the coordination sphere (Gentry et al., 2005). Consequently there is a fivefold decrease in affinity of EF3 (Asp12) compared to EF4 (Glu12) of CIB (calcium and integrin binding protein) (Amniuk et al., 2004).

The quantum of the favorable entropy term in eqn (3) can be decreased if unfavorable factors have a role to play in the entropy of Ca^{2+} binding to EF-hands. There are enough evidences to show that to bring a change in conformation of EF hand loop more energy is spent in comparison to the others. Both crystallographic B-factors and NMR relaxation give evidences that a glycine triplet in the *N*-terminal part of the EF1 loop of sTnC is more disordered, and consequently more stable, in terms of conformational entropic free energy, than EF2 (Li, E., et al., 1995; Strynadka et al., 1997). The expenditure of energy in bringing about an order in this flexible loop is reflected in its tenfold lower affinity compared with EF2 (Gagne et al., 1997). If there is an availability of 'preformed' EF-hands, such as the ψ- hand of calbindin D9K, the entropy cost to bring about an order in the loop is reduced and consequently there is an increase in the calcium affinity of this site (Ke et al., 1991). There is every possibility that EF-hands put to use such conformational entropy costs due to loop flexibility in the apo form to modulate their Ca^{2+} affinity (Agne et al., 1998).

A second unfavourable entropic factor is the Ca^{2+}-induced exposure of hydrophobic surfaces, as experienced by the Ca^{2+} sensors, an energy expensive process (Foguel et al., 1996). The amount of energy consumed to expose the hydrophobic surface is considerable and is evident from the reduction in the calcium affinity. This consumption of energy act as an opposite to the favourable release of water molecules (Nelson et al., 1998).Closed compact structure of calcium sensors does not permit calcium to bind to EF hand. For the calcium binding to happen it has to come in the open conformation. If the calcium could be accommodated in the closed structure, there would be stronger binding of the calcium to the EF hand loop.

These are some of the reasons which may provide a greater platform for the better understanding of the variation in calcium binding affinity of EF hands.