3.1: Ca^{2+}-binding Proteins in Microorganisms- The Search for a Prokaryotic Calmodulin

Since Ca^{2+} ions evidently play an important role in regulating a variety of cellular responses in animals and higher organisms, one may ask whether this use of Ca^{2+} is a recent discovery of Nature, or if it was invented early in evolution. It now appears well-established that the key intracellular "Ca^{2+}- receptor" protein calmodulin (CaM; see Section V.A) is present in all eukaryotic cells. Even in a unicellular eukaryote like common yeast (*Saccharomyces cerevisiae*), Ca^{2+} has an important regulatory role, and recently yeast CaM, as well as the single-gene encoding for it, was isolated.[160]

The amino-acid sequence of the yeast CaM (147 a.a.; M_r = 16.1 kDa) is 60 percent identical with the sequences of all other CaMs known. In fact, if generally accepted conservative amino-acid replacements are allowed, the homology increases to 80 percent or more, the most highly conserved portions being the four putative Ca^{2+}-binding sites. Sites I and III match the EF-hand test sequence (see Figure 3.24) very well; in site a His occurs after the "z"- ligand instead of the archetypal Gly; and in site IV there is no amino acid between the residues that usually make up ligands "x" and "y." The effect of these alterations on the Ca^{2+} affinity of yeast CaM is not yet known.

That CaM is essential for the growth of yeast cells was shown by deletion or disruption of the gene. This constitutes, in fact, the first demonstration in any organism that CaM is an essential protein. (Deletions of genes in mammals are ethically questionable research procedures!)

In the biochemically less sophiscated (than eukaryotes) **prokaryotic** cells, a regulatory role of Ca^{2+} is not well-established. What is known is that calcium is massively accumulated during sporulation in many bacteria, for example, in strains of *Bacillus*, *Streptomyces*, and *Myxococcus*. In *Myxococcus xanthus* a development-specific protein called protein S assembles at the surface of myxospores in the presence of Ca^{2+}. The DNA sequence of the gene that encodes this protein has been deciphered.[161] The primary sequence of protein S (175 a.a., M_r = 19.2 kDa) turns out to closely resemble mammalian CaM. It has four internally homologous regions with putative Ca^{2+} sites. At least two of these are partly similar to the typical EF-hand, but uncharacteristically there are many more prolines in the *M. xanthus* protein than in bovine CaM (12 versus 2); so it is questionable if the bacterial protein really has the repeated helix-loop-helix structure found in mammalian CaM.[162]

One candidate for a prokaryotic CaM was reported by Leadlay *et al.*[163] in *Streptomyces erythreaus*, the bacterium that produces the well-known antibiotic "erythromycin." The amino-acid sequence of a low-molecular-weight Ca^{2+}- binding protein, as determined from the gene encoding it, revealed a high homology with mammalian CaM. The protein is made up of 177 amino acids (M_r = 20.1 kDa), and has four regions that are predicted to have the helix-loop-helix secondary structure typical of EF-hand proteins. The aligned sequences of the 12 residues in each of the four potential calcium-binding loops in the *S. erythreaus* protein are compared with those of human calmodulin in Table 3.6.

Table 3.6 - Aligned EF-hand sequences for the prokaryotic and human calmodulins

Ligands	1		3		5		7		9			12
S. erythraeus protein I	D	F	D	G	N	G	A	L	E	R	A	D
S. erythraeus protein II	G	V	G	S	D	G	S	L	T	E	E	Q
S. erythraeus protein III	D	K	N	A	D	G	Q	I	N	A	D	E

Ligands	1		3		5		7		9			12
S. erythraeus protein IV	D	T	N	G	N	G	E	L	S	L	D	E
Human calmodulin I	D	K	D	G	D	G	T	I	T	T	K	E
Human calmodulin II	D	A	D	G	N	G	T	I	D	F	P	E
Human calmodulin III	D	K	D	G	N	G	Y	I	S	A	A	E
Human calmodulin IV	D	I	D	G	D	G	Q	V	N	Y	E	E

The pattern of residues in the *S. erythraeus* protein is typical of an EF-hand at least in sites I, III, and IV. Site II is unusual in having Gly at both positions 1 and 3. ^{113}Cd NMR studies show that the bacterial protein binds three metal ions strongly ($K \gtrsim 10^5\ M^{-1}$) with chemical shifts close to those expected for EF-hands, and ^{1}H NMR studies show that it undergoes a Ca^{2+}- dependent conformational change.[164]

Although the *S. erythraeus* protein has a homology with eukaryotic CaM, it has been pointed out that the protein has an even higher homology with a group of eukaryotic sarcoplasmic Ca^{2+}-binding proteins[165] (see Section V.D). The search for a prokaryotic CaM analogue continues, and the prospect of success has been improved after recent reports of a 21-amino-acid-Iong polypeptide from an *E. coli* heat-shock protein[166] that shows the typical structural features of CaM-binding domains in other eukaryotic proteins.[167]