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Ca^{2+} ion as a fluorescence probe to study the sites of Ca^{2+} binding in calmodulin

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Calmodulin (CaM) is a Ca^{2+} binding protein that plays an important role in cellular regulation (Cheung 1980)[1] . This protein was first identified by Kakiuchi and conveders(1970)[2] . It is known today that Ca^{2+} acts as a mediator in many C_i^{2+} ion dependent proteins.The first of such proteins was calmodulin.Calmodulin acts as the activator of enzymes such as phosphodiesterase (Cheung 1980)[1,3] and adenylate cyclase (Brostrom et al.,1975)[4].In both processes the dependency on Ca^{2+} ion as a divalent cation was established CaM is a single stranded protein with 148 amino acid and a molecular weight of 16,700 D.1/3 of amino acids being glutamate and asnartate.Those groups are known to be the main Ca^{2+} ion binding sites regi Cystein and tryptophan are absent in CaM,with only one histidine in the structure of protein.The high ratio of phenylalanine to tyrosine(4/1)has specific effect on protein extinction coefficient.The ratio of acidic amino acids to the basic ones are high(2.7)and hence the PI is low(PI=3.9).The two tyrosine of 138 and 99 in CaM are responsible for tyrosine fluorescence properties.[5] CaM contains four similar regions in its structure with one Ca^{2+} ion attached to each site[6,7] hexacoordinated to six amino acids.[3] Since the role of Ca^{2+}ion in modulation of the activation properties of CaM for cellular processes are important ,We tried to study the modes of binding of Ca^{2+} ion with respect to the specific groups that may be involved in bindings and the environment of such bindings.We used an already tested method devised in this laboratory to identify functional groups that are attached to metal ions in proteins and enzymes.This method has been worked out for some enzymes and proteins that are dependent on metal ions for their activities(Farzami 1990-1992)[8,9,10] In present article a fluorescent method was used to carry out this investigation. the calcium binding sites were determined by the difference fluouescence of apo-CaM and holo-CaM.With this study we hope to provide an understanding towards