Elucidation of pK_a values for Ca^{2+} binding sites in calmodulin by spectrofluorometry

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Received 23 March 1994

Calmodulin (CaM) was purified from bovine brain and identified on the basis of its phosphodiesterase activity. Its purity was further tested by electrophoretic migration in polyacrylamide gels in the presence of sodium dodecyl sulfate. Apo-CaM was prepared from holo CaM using hydroxyapatite chromatography. The Ca^{2+} binding sites on CaM and the pK_a of each of the functional groups bound to Ca^{2+} were identified from the dependence of Ca^{2+} interaction with the functional group as a function of pH.

EGTA was found to diminish the peaks corresponding to the pK_a values of the groups bound to Ca^{2+}. The use of bromophenacyl bromide, a modifier for aspartate and glutamate residues in proteins, diminished the peaks at pH = 3.4 and 4.3. Diethyl pyrocarbonate, a modifier for histidine residues, reduced the peak at pH = 6.2, corresponding to the pK_a of the imidazole group in histidine. Furthermore, the peak at pH = 11.6 was eliminated using the specific tyrosine modifier, *N*-acetylimidazole. Diethylpyrocarbonate also eliminated four small peaks at pH = 7.2, 7.8, 8.2 and 8.8. This effect could be attributed to the binding of threonine and serine residues. The crystallographic results for parvalbumin, which has a similar molecular structure, suggest identical Ca^{2+} binding sites.

Keywords: calmodulin; calcium binding; pK_a determination

Calmodulin (CaM) is a Ca^{2+} binding protein that plays an important role in cellular regulation[1]. It was first identified by Kakiuchi and Yamazaki[2]. It is now known that Ca^{2+} also acts as a mediator in many other Ca^{2+} ion-dependent proteins. CaM acts as the activator of enzymes such as phosphodiesterase[1,3] and adenylate cyclase[4]. In both processes, the dependency on the Ca^{2+} ion as a divalent cation has been established. CaM is a single-stranded protein with 148 amino acids and a molecular weight of 16 700. One-third of the amino acids are glutamate and aspartate, which are known to be the main Ca^{2+} binding sites. Cysteine and tryptophan are absent in CaM, with only one histidine present. The high ratio of phenylalanine to tyrosine (4/1) has a specific effect on the protein extinction coefficient. The ratio of acidic to basic amino acids is high (2.7), and hence the p*I* is low (p*I* = 3.9). The two tyrosines (at positions 138 and 99) are responsible for the 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 the Ca^{2+} ion is important in modulating the activation properties of CaM for cellular processes, we have studied the modes of binding of the Ca^{2+} ion with respect to the specific groups that may be involved in binding and the environment of such binding. We used a tested method devised in this laboratory to identify functional groups that are attached to metal ions in proteins and enzymes. This method was determined for certain enzymes and proteins that are dependent on metal ions for their activities[8-10]. In this paper, a fluorescent method was used to carry out this investigation, and the Ca^{2+} binding sites were determined by the difference fluorescence of apo-CaM and holo-CaM. We hope that this investigation will provide an understanding of the binding of the metal ion to each site in CaM, together with details of the environment of each site.

Experimental

Materials

Diethylaminoethyl (DEAE)-Sepha... was obtained from Pharmacia (Sweden). Hydroxyapatite was prepared

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0141-8130/94/040161-06