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Calcium-induced calmodulin conformational change. Electrochemical evaluation

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ABSTRACT

Calmodulin (CaM) is an essential protein present in all eukaryote cells, ranging from vertebrates to unicellular organisms. CaM is the most important Ca^{2+} signalling protein, composed of two domains, N- and C-terminal domains, linked by a flexible central α-helix, and is responsible for the regulation of numerous calcium-mediated signalling pathways. Four calcium ions bind to CaM, changing its conformation and determining how it recognizes and regulates its cellular targets. The oxidation mechanism of native and denatured CaM, at a glassy carbon electrode, was investigated using differential pulse voltammetry and electrochemical impedance spectroscopy. Native and denatured CaM presented only one oxidation peak, related to the tyrosine amino acid residue oxidation. Calcium-induced calmodulin conformational change and the influence of Ca^{2+} concentration on the electrochemical behaviour of CaM were evaluated, and significant differences, in the tyrosine amino acid residue peak potential and current, in the absence and in the presence of calcium ions, were observed. Gravimetric measurements were performed with a graphite coated piezoelectric quartz crystal with adsorbed CaM, and calcium aggregation by CaM was demonstrated.

1. Introduction

Calmodulin (CaM) is a small protein present in all eukaryote cells, ranging from unicellular organisms to vertebrates. CaM is the most important calcium signalling protein, involved in the regulation of numerous Ca^{2+} dependent pathways [1,2].

CaM is essential in all organisms due to the broad spectrum of functions in the control of numerous physiological processes: synthesis and release of neurotransmitters, regulation of intracellular calcium concentration, cell motility and proliferation, apoptosis, autophagy, metabolic homeostasis, protein folding, ions transport, osmotic control, reproductive processes, muscle contraction and gene expression, among others [2,3].

CaM is composed of two domains, N- and C-terminal domains, linked by a flexible central α-helix (Scheme 1) [4]. The N-terminal domain contains EF-hands, EF-1 and EF-2, called CaM_{12} and the C-terminal domain contains EF-hands EF-3 and EF-4, called CaM_{34} [5]. The EF-hand structural motif was devised in 1973 and is the most common calcium-binding motif found in proteins [6].

The EF-hand motif contains a helix-loop-helix topology, much like the thumb and forefinger of the human hand, each representing a helix and the middle finger bent to suggest the octahedral loop in which the Ca^{2+} ions are coordinated by ligands within the loop region (usually about 12 amino acids). The EF-hand motif contains approximately 40 residues and is involved in binding intracellular calcium [6].

The CaM can bind to four calcium ions (two Ca^{2+} per domain) and exists in two forms: without calcium (ApoCaM) and in the calcium saturated form (HoloCaM) [3,5]. Increasing calcium intracellular levels, Ca^{2+} binds to CaM and undergoes an "open form" conformational change with the hydrophobic structure exposed [5].

Each domain of the Ca^{2+} saturated HoloCaM contains a methionine-rich cavity. The flexibility and polarizability of the methionine amino acid residues, present on the hydrophobic surface and other hydrophobic amino acid residues, enable the occurrence of adjustable interaction areas that can aggregate CaM targets [5,6]. CaM is able to bind to a large array of peptides, enzymes, protein myosin kinase, protein kinase II and others, with different sizes and shapes, and modulate their activity in many different ways, leading to biochemical and cellular changes [6,7].

The Ca^{2+} binding to CaM is not always essential for some of the vital roles and regulatory function of CaM. ApoCaM also binds and can regulate a variety of proteins, including neuromodulin, neurogranin, unconventional myosins and enzymes that can interact with CaM, in the absence or for very low Ca^{2+} concentrations [1].

CaM-dependent signalling mechanisms involved in cell proliferation, programmed cell death or autophagy, are essential in tumour cell biology [2,8–10]. Some studies have demonstrated anomalous intracellular concentrations of CaM and other Ca^{2+} binding proteins in tumour cells, compared with cells from normal tissues [2].

CaM has also been considered a crucial molecule in the etiology of adolescent idiopathic scoliosis and some studies suggest that platelet

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Nomenclature

CaM	(calmodulin)
Tyr	(tyrosine)
Trp	(tryptophan)
His	(histidine)
Cys	(cysteine)
Met	(methionine)
SDS	(sodium dodecyl sulphate)
DTT	(dithiothreitol)
TCEP	(tris(2-carboxyethyl)phosphine)
GCE	(glassy carbon electrode)
CV	(cyclic voltammetry)
DP	(differential pulse)
P_{Tyr}	(tyrosine oxidation peak)
EIS	(electrochemical impedance spectroscopy)
EQCM	(electrochemical quartz crystal microbalance)
CPE	(constant phase element)

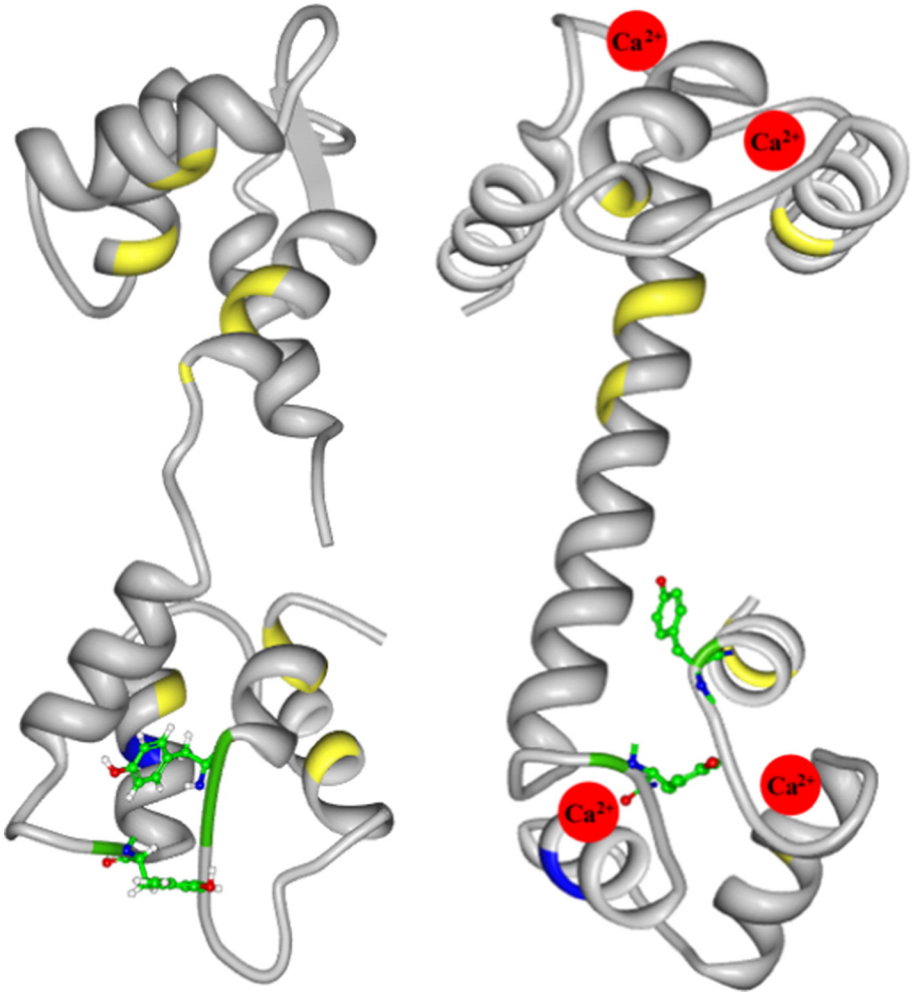
CaM levels may be a better biomarker for the disease curve progression [11,12].

The improvement of effective methods for the determination and quantification of CaM is essential for drug development, clinic diagnosis and disease etiology research due to the importance of CaM in the control of numerous physiological processes. There are some biochemical, thermodynamic and structural studies about CaM and the Ca^{2+}-induced CaM conformational changes [13–17].

Investigation of the electrochemical behaviour and the protein interactions with solid electrode surfaces is very important in drug discovery and a key to novel applications in biosensors, biotechnology and medical devices. The electrochemical study of proteins has been the main subject of many scientific papers over recent years, using gold and carbon electrodes [18–21]. From the 20 amino acids present in proteins, the oxidation only occurs in five electroactive amino acids: tyrosine (Tyr), at E_p ~ 0.65 V, tryptophan (Trp), at E_p ~ 0.65 V, histidine (His), at E_p ~ 1.1 V, cysteine (Cys), at E_p ~ 0.55 V, and methionine (Met), at E_p ~ 1.25 V [22]. At carbon electrodes these amino acids are oxidized in an irreversible pH-dependent electron transfer mechanism [23–25].

The CaM structure is formed by a sequence of 148 amino acids, and the electroactive amino acids are methionine (10 residues), tyrosine (2 residues) and histidine (1 residue) (Scheme 2). Tyrosine undergoes oxidation at glassy carbon electrodes and at boron doped diamond electrodes, with one-irreversible peak corresponding to the oxidation of the hydroxyl group [19]. Methionine oxidation occurs in two steps involving the adsorption and the protonation/deprotonation of the thioether group [19,23,24]. Histidine oxidation occurs at a higher positive potential in a single step [22,25].

In the present work, the influence of Ca^{2+} on the CaM conformational change, and the electrochemical oxidation behaviour of native and



Scheme 1. 3D Structure of Apo-Calmodulin (left) and Holo-Calmodulin (right) with calcium atoms (red) and the electroactive residues, methionine (yellow), tyrosine (green) and histidine (blue) [4]. (For interpretation of the references to color in this scheme legend, the reader is referred to the web version of this article.)