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Preparation of Europium Induced Conformation-specific anti-calmodulin Monoclonal Antibody

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Abstract: Monoclonal antibody technique was employed to detect the conformational difference of CaM induced by metal ions. A trivalent europium ion induced conformation-specific anti-calmodulin monoclonal antibody was successfully prepared with europium-saturated calmodulin as antigen.

Keywords: Calmodulin, europium, monoclonal antibody.

Calmodulin (CaM), a ubiquitous calcium-binding protein in eucaryotic cells, serves as a multifunctional regulator in variety of cellular processes and regulates the activities of more than thirty enzymes and proteins[1]. CaM has four calcium-binding sites, when it is saturated with calcium ions, the protein undergoes conformational changes with exposure of its hydrophobic residues at central linker, which allows the protein to interact with its target enzymes. It has been reported that compared with calcium ion, trivalent lanthanide ions showed an inhibitory effect on the activities of CaM′s target enzymes, probably due to the facts that, after binding with CaM, calcium and lanthanide ions induce different conformational changes of CaM. Results from UV and CD spectra revealed that lanthanide ions bind to CaM at the same calcium-binding sites with decrease of α-helix content of the protein′s secondary structure[2,3], but there is still little information about the tertiary structure changes of CaM induced by lanthanide ions.

Monoclonal antibody (McAb) is a sensitive technique for evaluating molecular recognition abilities between antibody and antigen, there have been some reports on protein interactions by this technique[4]. In this paper, McAb was employed to detect the conformational difference of CaM induced by metal ions, and a trivalent europium ion induced conformation-specific McAb was successfully prepared.

A bovine brain CaM was isolated and purified with purity of more than 99% confirmed both by SDS-PAGE and MALDI-TOF mass spectrometry. Calcium ions contained in the protein were removed by a method of ultrafiltration with calcium chelator and protein without calcium (apoCaM) was prepared. Because CaM is a small acidic protein with poor immunogenicity, the protein was firstly modified by 2, 4-dinitrofluorobenzene in order to improve hydrophobicity of its residues. The protein

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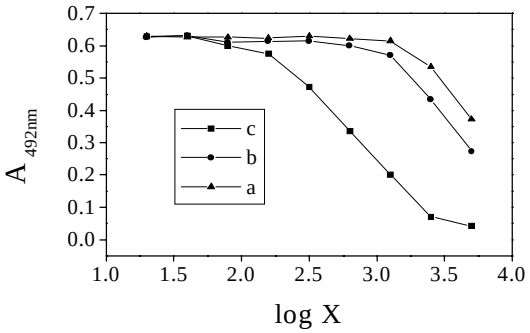
(DNB-CaM) saturated with europium ions was injected to mice as antigen, after four times of immunization, antibody titer in serum was detected as 1: 12000. The spleen cells of immunized mice were fused with hybridoma cells successfully, and one cell strain named as 2C3 was produced as normal. Ascitic fluid was obtained and purified by affinity chromatography.

Figure 1 demonstrates preliminary results about the immunological response of antibody to different types of antigens measured by a method of ELISA. Binding of antibody to DNB-CaM is tighter (**a)** than to europium-saturated CaM (**b**), while apoCaM shows week recognition with antibody (**c**), indicating that apoCaM undergoes a conformational change, which is different from that of apoCaM after binding with europium ions. The results revealed that antibody is a sensitive technique for investigating CaM′s conformational changes induced by metal ions.

Figure 1 Binding of antibody to antigens



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