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论文

钙调素与重金属Pb^{2+}结合反应的方波极谱与循环伏安法研究

刘德龙[1], 吴彦环[1], 郭慧芳[2], 白娟[2], 孙大业[2]

1. 河北师范大学化学学院,
2. 生命科学学院, 石家庄 050016

摘要:

采用方波极谱法研究了重金属Pb^{2+}与钙调素(CaM)的结合反应, 直接检测到Pb^{2+}-CaM配合物的存在, 并进一步利用循环伏安法研究了Pb^{2+}-CaM的电极反应. 在pH=6.5时, 用方波极谱法在Pb^{2+}-CaM体系中检测出2个还原峰, 峰电位分别为-0.44~-0.47 V和-0.73~-0.77 V, 说明在Pb^{2+}-CaM体系中铅有2种存在形式, -0.44~-0.47 V的还原峰对应于游离态Pb^{2+}, 电位更负的还原峰对应于配合物[Pb^{2+}-CaM]. 2个还原峰的峰电流均随着c_{Pb2+}/c_{CaM}比值增大而增大; 至c_{Pb2+}/c_{CaM}≥10后, 配合物[Pb^{2+}-CaM]的峰电流基本不再变化, 而游离态Pb^{2+}的峰电流则继续增大. 利用极谱滴定曲线的拐点可判断出Pb^{2+}在CaM中有10个结合位点. 进一步的测量结果表明, 循环伏安曲线出现游离态Pb^{2+}的氧化峰和还原峰, 而络合态的[Pb^{2+}-CaM]只有其还原峰, 反向电压扫描时不出现阳极波, 即没有相对应的氧化峰出现.

关键词: 钙调素; 重金属离子Pb2+; 结合位点; 方波极谱法; 循环伏安法

Direct Binding of Reaction Pb^{2+} to Calmodulin by Square Wave Polarography and Cyclic Voltametry

LIU De-Long[1*], WU Yan-Huan[1], GUO Hui-Fang[2], BAI Juan[2], SUN Da-Ye[2]

1. College of Chemistry,
2. College of Life Science, Hebei Normal University, Shijiazhuang 050016, China

Abstract:

Calmodulin(CaM) is a highly conserved Ca^{2+} binding protein ubiquitously found in animals and plants, which is involved in a large variety of cellular functions. The presence of many other metal ions in the physiological and nonphysiological environment such as heavy metal ions suggests that CaM might be binding other metal ions than Ca^{2+}, which might influnce CaM's function. It is important to investigate the general metal ion binding properties of CaM. Based on high sensitivity of square wave polarographic signal of Pb^{2+}, the direct binding reaction of Pb^{2+} to CaM was studied by square wave polarography (SWP). The complexing specie, Pb^{2+}-CaM, was detected for the first time by SWP in the Pb^{2+}-CaM system, and electrochemical reaction characterization was done by cyclic voltammetry. Two reduction peaks were detected in SWP polarograms obtained at different concentration ratios of Pb^{2+} to CaM at pH=6.5, indicating that two electroactive species of Pb^{2+} exist, the reduction peak potentials of two species are in the range of -0.44—-0.47 V and -0.73—-0.77 V *vs*. SCE, respectively. The peak with a maximum at *ca*. -0.44—-0.47 V is corresponding to the reduction of free Pb^{2+} under our experimental conditions and the peak with a maximum at *ca*. -0.73—-0.77 V, clearly more negative than that for the reduction of free Pb^{2+}, allows us to interpret it as due to the reduction of Pb^{2+} complexed by CaM. Moreover, prior to the addition 10 times of Pb^{2+}, two peak currents increase gradually with the increasing of the Pb^{2+} concentration. At higher metal ion concentration(10—16 times), the peak currents of free Pb^{2+} increased linearly with a higher value of the slope, while the peak currents of the complexing specie, Pb^{2+}-CaM, reached maximal and constant. The polarographic titration curves of the two species show that there are ten binding sites in CaM at pH=6.5. Furthermore, the reduction species of Pb^{2+}-CaM system was confirmed by cyclic voltammetry with Controlled Growth Mercury Electrode (CGME). One couple of the free Pb^{2+} redox waves were observed clearly in the cyclic voltammogram, and only the reduction peak of the complexing specie of Pb^{2+}-CaM system was detected. The results obtained in the paper show a direct evidence for the mechanism of the toxicity of Pb^{2+} by CaM mediating.

Keywords: Calmodulin; Heavy metal ion Pb^{2+}; Binding site; Square wave polarography; Cyclic voltammetry

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通讯作者: 刘德龙, 男, 博士, 教授, 主要从事生物分析化学与化学生物学方面研究. E-mail: delongliu9012@sina.com

作者简介:

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