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铽-钛铁试剂络合物荧光探针研究辅酶及其表面活性剂增敏效应

Study on Coenzymes by Tb^{3+}-Tiron Complex Fluorescence Probe and Surfactant Effect on the Probe

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摘　要

辅酶是一类具有特殊化学结构和功能的化合物，它与酶蛋白组成结合蛋白酶催化生物体内进行的氧化还原反应或基团转移反应。随着生命科学及酶学的发展，需要更加灵敏的辅酶测定方法，用于表征结合蛋白酶的催化动力学行为和酶的活性，本文就是为这一目的而进行的基础研究。本论文共分为四章。

第一章综述了稀土络合物荧光探针的特征、分类及在生命科学中的应用，重点讨论了铽－钛铁试剂（Tb^{3+}-TR）络合物荧光探针的特点和应用，介绍了辅酶的特征、几种重要辅酶及其文献报道的测定方法，阐述了表面活性剂胶束增敏荧光的相关理论。在文献综述的基础上提出了本论文的研究设想。

第二章考察了含磷酸基辅酶对Tb^{3+}-TR络合物荧光的猝灭作用。推导了静态荧光猝灭法中猝灭剂的初始浓度与荧光猝灭值ΔF的关系式，选取磷酸吡哆醛（PALP）、辅酶Ⅰ(NAD)、辅酶Ⅱ(NADP)对该关系式进行了验证，并建立了以Tb^{3+}-TR络合物为荧光探针的荧光猝灭法测定含磷酸基辅酶的新方法。

第三章考察了溴化十六烷基三甲铵（CTMAB）等六种表面活性剂对Tb^{3+}-TR络合物荧光的影响，初步探讨了表面活性剂胶束对络合物荧光的作用机理，实验表明，CTMAB可以增强Tb^{3+}-TR络合物荧光，预示着CTMAB-Tb^{3+}-TR体系可作为荧光猝灭法测定辅酶的高灵敏体系。

第四章建立了表面活性剂增敏Tb^{3+}-TR络合物荧光猝灭法测定含磷酸基

辅酶的高灵敏方法。详细考察了PALP、NAD、NADP和黄素腺二核苷酸(FAD)四种重要辅酶的最佳测定条件，测定的线性范围分别为 2.8×10^{-8}～9.2×10^{-7} mol/L、1.0×10^{-8}～3.6×10^{-7} mol/L、1.2×10^{-8}～1.8×10^{-7} mol/L和 1.0×10^{-8}～2.6×10^{-7} mol/L，检出限分别为 1.81×10^{-8}mol/L、6.46×10^{-9}mol/L、5.33×10^{-9}mol/L和 7.01×10^{-9}mol/L。

关键词：铽一钛铁试剂络合物，辅酶，荧光增敏，荧光猝灭

ABSTRACT

Coenzymes are a kind of compounds with special chemistry structures and functions. They could combine with apoenzyems to constitute conjugated proteases which catalyze the reactions of redox and group transfer. As the life sciences and the enzymology advanced, it is necessary to develop novel sensitive methods for the determination of coenzymes in order to detect some chemical properties of conjugated proteases and their dynamics behavior. This dissertation is about one foundation research for this purpose, which consists of four chapters.

In chapter one, the characteristics, classifications and applications in life sciences of rare-earth ionic complex as fluorescence probes were summarized, and especially discussed the characteristics and the applications of Tb^{3+}- Tiron complex. The characteristic of coenzyme were also introduced, and several important coenzymes and their determination methods reported in literature were viewed. At last, the correlative theoretics of the effect of surfactant micelle on the fluorescence intensity were expounded. Based on that the research conceives were put forward.

In chapter two, the fluorescence quenching of Tb^{3+}-Tiron complex by coenzymes containing phosphate groups was studied. Theoretically, the quantitative formula on the relationship between the initial concentration of quencher and the quenching value of fluorescence intensity, $\triangle F$, was induced. Furthermore, three coenzymes (PALP, NAD, NADP) were chosen to validate the formula. A new method was developed for the determination of these coenzymes.

In chapter three, the effect of six kinds of surfactants on the fluorescence of Tb^{3+}-Tiron complex was studied respectively. The experiment indicated that CTMAB increasing the fluorescence intensity of Tb^{3+}-Tiron complex, and

indicated that CTMAB- Tb^{3+}- Tiron system can be used as a novel sensitive system to detect the coenzymes containing phosphates based on the fluorescence quenching of Tb^{3+}-Tiron complex.

In chapter four, novel sensitive methods for the determination of PALP, NAD, NADP and FAD which contain phosphate groups were developed. The methods based on the fluorescence quenching of CTMAB-Tb^{3+}-Tiron system by PALP, NAD, NADP and FAD, respectively. The optimal conditions of the fluorescence quenching of CTMAB-Tb^{3+}- Tiron system were studied. Under the optimal conditions, PALP, NAD, NADP and FAD were determined quantitively. The linear ranges of them are 2.8×10^{-8}～9.2×10^{-7} mol/L、 1.0×10^{-8}～3.6×10^{-7} mol/L、 1.2×10^{-8}～1.8×10^{-7} mol/L and 1.0×10^{-8}～2.6×10^{-7}mol/L， respectively. The detection limits are 1.81×10^{-8} mol/L、6.46×10^{-9}mol/L、5.33×10^{-9}mol/L and 7.01×10^{-9}mol/L， respectively.

Key words: Tb^{3+}-Tiron complex, coenzyme, fluorescence enhancement, fluorescence quenching

目 录

CONTENT

第一章　前 言

1.1 引言

荧光探针在科学技术中的应用可追溯到很久以前，人们利用荧光染料来探测水源或用它来做为海难中营救的识别标志。近几十年来，随着光化学和光物理研究的进展、荧光检测技术水平的提高及对发光行为机理的深入了解，这一技术在科学研究中的应用得以迅速发展。荧光探针技术是利用荧光探针化合物的光物理和光化学特征，在分子水平上研究某些体系物理或化学过程的机理、动力学以及某些特殊环境的物化特性或某种化合物的结构、构象及其物化性质的方法。稀土络合物由于其独特的荧光性能，在荧光探针研究中得到了广泛的应用。

1.2 稀土络合物荧光探针

1.2.1 稀土络合物荧光探针的特征

稀土离子吸收了来自紫外光、电子射线等的辐射能后，可通过三种跃迁之一由基态变为相应的激发态，再以非辐射衰变至 $4f^n$组态的激发态（亚稳态），此能态以辐射跃迁便产生稀土荧光。这三种跃迁是[1]：(1)来自f^n组态内能级间的跃迁（f→f跃迁）； (2)组态间能级的跃迁（f→d跃迁）；(3)配体向稀土离子的电子跃迁（电荷转移跃迁）。

从电子结构来看，稀土的荧光性能可分为三类，如表 1.1 所示。

表 1.1　稀土荧光性能（稀土离子的一般电子构型为$[Xe]4f^n5s^25p^6$）

稀土离子	电子结构	荧光性能
Sc^{3+}	$[Ar]3d^0$	不产生荧光：4f 没电子或全充满，没有 4f 到 4f 跃迁。在紫外光区和可见光区均无吸收。
Y^{3+}	$[Kr]4d^0$	
La^{3+}	$[Xe]5d^0$	
Lu^{3+}	$[Xe]4f^{14}$	