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厦 门 大 学

硕 士 学 位 论 文

生物大分子与小分子相互作用的静态荧光猝灭理论及实验研究

Theoretical and Experimental Study of Fluorescent Static Quenching for the action of Macrobiological Molecule with Micromolecule

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专　业　名　称：分　析　化　学

论文提交日期：2005年6月

论文答辩时间：2005年7月

学位授予日期：

答辩委员会主席：_____

评　　阅　　人：_____

2005 年　　月

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Theoretical and Experimental Study of Fluorescent Static Quenching for the action of Macrobiological Molecule with Micromolecule

A Dissertation Presented

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Summited to the Graduate School of Xiamen University

for the Degree of

MASTER OF SCIENCE

July, 2005

Department of Chemistry, Xiamen University

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摘要

小分子静态荧光猝灭理论是生成化合物荧光猝灭法测定的基础，但常被应用于生物大分子的定量测定，然而小分子静态荧光猝灭理论须满足：猝灭剂（Q）与荧光体反应生成 1：1 的配合物，且生成的配合物不发荧光，因此其推导结果$\frac{F_0}{F}=1+K[Q]$对大多数小分子间的相互作用是适用的，但对于小分子与生物大分子间的相互作用就不再适用。原因是生物大分子与荧光体小分子作用时，它们的结合往往不再是简单的 1:1 关系,而是一个生物大分子可结合 n 个荧光体小分子，而且在一些情况下，随着生物大分子和小分子相对浓度的变化，结合比是变化的。同时需要指出的是，上式中的[Q]为反应后猝灭剂的平衡浓度，而非猝灭剂的初始浓度$[Q]_0$，由于实际测定时初始浓度$[Q]_0$是已知的，而平衡浓度[Q]通常难以测定，故上式不能用作定量测定的依据。在实际测定时通常混淆了$[Q]_0$与[Q]的差异，以$[Q]_0$替代[Q]是不科学的。近几年来，研究者在相关研究中，已经意识到结合位点的问题，在相关理论研究时考虑了生物大分子与小分子之间相互作用时 1：n 的结合比关系，但仍忽略了猝灭剂的平衡浓度[Q]与初始浓度$[Q]_0$的差异，因而在应用时是不科学的。由于现有的静态荧光猝灭理论存在以上不足，所以对静态荧光猝灭理论的研究具有重要的科学意义和实用价值。本论文共分四章。

第一章综述了荧光猝灭的定义、分类及其导致荧光猝灭的原因，重点介绍了静态荧光猝灭的分类及现在所存在的静态荧光猝灭理论。在评述现阶段理论的基础上，指出了现有的荧光猝灭理论存在的两大不足，并提出了本论文的研究设想。

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第二章研究了生物大分子内源荧光探针的荧光猝灭理论，探讨了生物大分子内源荧光探针与小分子之间相互作用的静态荧光猝灭理论，推导了它们之间相互作用的表观结合常数 K 和结合位点数 n 的数学表达式，及其相关的定量测定关系式。推导结果表明，荧光体的荧光强度与猝灭剂的相互关系与荧光体和猝灭剂之间的相对浓度有直接的关系，在猝灭剂的初始浓度$[Q]_0$远大于荧光体的初始浓度时，$\lg\left(\frac{F_0-F}{F}\right)=\lg K+n\lg[Q]_0$；而在猝灭剂的初始浓度$[Q]_0$远小于荧光体的初始浓度时，$\Delta F=k\frac{n^2}{n_t}[Q]_0$。本文对上述理论推导进行了实验验证，结果表明，$\Delta F$ 和 $\lg\left(\frac{F_0-F}{F}\right)$分别与猝灭剂初始浓度$[Q]_0$和$\lg[Q]_0$在一定浓度范围内呈正比，与理论推导的结果一致。该理论和实验结果克服了现有的荧光猝灭理论和实验的不足，不仅建立了内源荧光探针静态荧光猝灭法测定生物大分子的更加科学的方法，而且对研究生物大分子与小分子荧光探针之间的相互作用具有一定的指导作用。

第三章研究了生物大分子对有机小分子外源荧光探针的静态荧光猝灭理论，推导了它们之间相互作用的表观结合常数 K 和结合位点数 n 的数学表达式，及其相关的定量测定关系式。研究表明，荧光体的荧光强度与猝灭剂的相互关系与荧光体和猝灭剂之间的相对浓度有直接的关系，在猝灭剂的初始浓度$[Q]_0$远大于荧光体的初始浓度时，$\lg\frac{1}{F}=\frac{1}{n}\lg\left(\frac{nK}{F_0k^{n-1}}\right)+\frac{1}{n}\lg[Q]_0$；而在猝灭剂的初始浓度$[Q]_0$远

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小于荧光体的初始浓度时，$\Delta F = nk[Q]_0$。论文对上述结果进行了实验验证，实验表明 ΔF 和 $\lg\left(\frac{1}{F}\right)$分别与猝灭剂初始浓度$[Q]_0$和$\lg[Q]_0$在一定浓度范围内呈正比，与理论推导的结果一致。

第四章研究了生物大分子对金属离子及其络合物外源荧光探针的静态荧光猝灭理论，推导了表观结合常数 K 和结合位点数 n 的数学表达式，及定量测定关系式。研究结果表明，荧光体的荧光强度与猝灭剂的相互关系与荧光体和猝灭剂之间的相对浓度有直接的关系，在猝灭剂的初始浓度$[Q]_0$远大于荧光体的初始浓度时，$\lg\left(\frac{F_0}{F}\right) = \frac{1}{n}\lg[Q]_0 + \frac{1}{n}\lg\left(\frac{nkK}{F_0}\right)$；而在猝灭剂的初始浓度$[Q]_0$远小于荧光体的初始浓度时，$\Delta F = nk[Q]_0$。论文对上述推导结果进行的实验验证表明，$\Delta F$ 和 $\lg\left(\frac{F_0}{F}\right)$分别与猝灭剂初始浓度$[Q]_0$和$\lg[Q]_0$在一定浓度范围内呈正比，比较套用小分子间的静态荧光猝灭关系式$\frac{F_0}{F} = 1 + K[Q]$所得的结果，前者具有更宽的线性范围，同时，前者考虑了$[Q]$与$[Q]_0$之间的区别，比套用小分子间静态荧光猝灭的相关表达式更具科学性。

关键词：生物大分子；小分子；荧光猝灭理论；实验验证

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ABSTRACT

The theory of the micromolecular fluorescent static quenching is the basic of the fluorescent quenching detection by compound formed, but it has always been applied for the quantitative determination of macrobiological molecules. However the theory of the micromolecular fluorescent static quenching should satisfy the following conditions: the compound formed between the quencher (Q) and the fluorescence probe is nonfluorescent, and the reacting locus ratio is 1:1. So the formula: $\frac{F_0}{F} = 1 + K[Q]$ which was deduced according to the above conditions was appropriate for the interaction between micromolecule and micromolecule but not for the interaction between micromolecule and macrobiological molecule. The reasons were that the reacting locus ratio between macrobiological molecule and micromolecule wasn't 1:1 but 1:n, and the reacting locus ratio might be alterable as the change of the relative concentration. Meanwhile, it had to emphasize that [Q] is the equilibrium concentration but not the initial concentration in the above formula. Owing to the initial concentration is known, whereas obtaining the equilibrium concentration is very difficult. When the determination is performed, the above formula is restricted in practice. Over the last several years, many researchers have been awared that the reacting locus ratio between macrobiological molecule and micromolecule wasn't 1:1, but still not awared the difference between the equilibrium concentration and the initial concentration in the existing formulas. Owing to the deficiencies of the existing theories of fluorescent static quenching, study

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on the theory of the fluorescent static quenching was necessary and significant. This dissertation is about one foundation research for this purpose, which consists of four chapters.

In chapter one，more than 80 references which include the definition、classification and mechanism of fluorescence quenching were reviewed, and especially discussed the classification and the existing theories of the fluorescent static quenching. According to the developments of the recent researches, two primary deficiencies of the existing theories of the fluorescent static quenching were presented，and the research conceives of this dissertation were proposed

In chapter two, the theory of fluorescent static quenching of the internal fluorescent probes was discussed. The relationship concerning the apparent formation constant K and the reacting locus number n were deduced and discussed, and the formulas of the quantitative determination were also deduced and proved by experiment. The deduced result as follows, $\Delta F = k\frac{n^2}{n_t}[Q]_0$ when the initial concentration of quencher is far less than the initial concentration of fluorescence probe, and $\lg\left(\frac{F_0 - F}{F}\right) = \lg K + n\lg[Q]_0$ when the initial concentration of quencher is far higher than the initial concentration of fluorescence probe. The experiment results showed that the signals, ΔF and $\lg\left(\frac{F_0 - F}{F}\right)$, are proportional to the initial concentration of quencher and $\lg[Q]_0$, respectively, which proved the theoretical results. To some extent, these

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theoretical and experimental results overcome the disadvantages of the existing theoretical results. So these results not only establish a scientific method of quantitative determination which can really describe the fluorescent static quenching, but also have instructions for the study on the action between micromolecule fluorescence quencher and macrobiological molecular internal fluorescent probes.

In chapter three, the interaction between small organic external fluorescent probes and macrobiological molecules were researched. The relationship concerning the apparent formation constant K and the reacting locus number n were deduced and discussed, and the formulas of the quantitative determination were also deduced and proved. The deduced result as follows, $\Delta F = nk[Q]_0$ when the initial concentration of quencher is far less than the initial concentration of fluorescence probe, and $\lg\frac{1}{F} = \frac{1}{n}\lg\left(\frac{nK}{F_0 k^{n-1}}\right) + \frac{1}{n}\lg[Q]_0$ when the initial concentration of quencher is far higher than the initial concentration of fluorescence probe. The experiment results showed that within the certain scope of concentration, ΔF and $\lg\left(\frac{1}{F}\right)$ are proportional to initial concentration of quencher and $\lg[Q]_0$, respectively, and the experiment results is consistent with the theoretical results.

In chapter four, the interaction between external fluorescent probes (metal ion or complex of metal ion etc.) and macrobiological molecule were researched. Uniformity, the relationship concerning the apparent

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formation constant K and the reacting locus number n were deduced and discussed. The deduced result as follows, $\Delta F = nk[Q]_0$ when the initial concentration of quencher is far less than the initial concentration of fluorescence probe, and $\lg\left(\frac{F_0}{F}\right) = \frac{1}{n}\lg[Q]_0 + \frac{1}{n}\lg\left(\frac{nkK}{F_0}\right)$ when the initial concentration of quencher is far higher than the initial concentration of fluorescence probe. The experiment results showed that the signals, ΔF and $\lg\left(\frac{F_0}{F}\right)$, are proportional to initial concentration of quencher and $\lg[Q]_0$, respectively, which proved the theoretical results, and the linear range was wider compared with the linear range of $\frac{F_0}{F} = 1 + K[Q]$. Moreover, the deduced formulas in this dissertation distinguished the equilibrium concentration from the initial concentration. So the results in this dissertation were rather scientific and reliable than the existing theoretical results.

Key Words: Macrobiological molecule; Micromolecule; Theory of fluorescence quenching; Experimental testification

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第一章 前 言

1.1 引言

分析化学学科自身的进步及与其它学科的交叉发展，尤其是近年来现代分析仪器和计算机技术的联用，为拓展药物分析的应用领域打下了良好的基础。荧光分析因具有高灵敏度、高选择性、信息量丰富、检测限低等特点，广泛用于生物化学、生物医学、临床分析等研究领域的痕量分析，以及从分子水平上对核酸、蛋白质、多肽等生物大分子及超痕量、超微量生物活性物质、单个细胞内神经传递物质（多巴胺、儿茶酚胺等）的分析等。这种分析技术不仅被用于进行物质的定性和定量分析，而且被用于测定生物大分子的构象，以及固态物质、生物大分子与有机分子和金属离子相互作用的研究，在临床医学、环境检测、法医鉴定、生命科学以及有序介质中生物大分子荧光探针光谱特征的研究等方面，发挥着极为重要的作用[1-4]。

随着荧光分析法的研究与应用的迅速发展，有关荧光分析法原理和应用的专著相继出版发行。荧光猝灭法的相关理论主要源自Lakowicz[5]或陈国珍[6]等的相关专著。

1.2 荧光猝灭

1.2.1 荧光猝灭的定义

荧光猝灭（或称荧光熄灭），广义上是指任何可使某种给定荧光物质的荧光强度下降的作用，任何可使荧光强度不与荧光物质的浓度成线性关系的作用，或任何可使荧光量子产率降低的作用。狭义

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