

滨州学院学术著作出版基金资助项目

Study on the Novel Near-infrared Fluorescent Probes Based on Indole Heptamethine Cyanine Dyes

Chunlong Sun

孙春龙 著



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The research work on the molecular structure alteration or improvement designed for increasing the stability and spectrum properties of indole heptamethine cyanine dyes has always been a concern. With indole heptamethine cyanine dyes as the parent, these novel indocyanines having good fluorescent imaging properties and photostability, can be used in fluorescent imaging analysis as fluorescent labeling reagents.

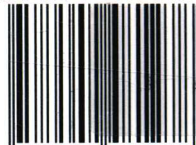
In this book included more spectral chart and table of Numbers, for the reader to see. And this book can provide reference for researchers engaged in chemistry, chemical engineering, biology, medicine, health, food and so on.



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Foreword

Molecular imaging is one of the most important topics in the fields of chemical biology, noninvasive diagnosis of diseases and nanomedicine. Over the past 10 years, efforts have also been directed toward the development of high-resolution, noninvasive, in molecular imaging technology. Now noninvasive molecular imaging technology has been developed energetically. Various magnetic resonance imaging techniques have prevalently application in clinic, but the existence of the spatial or temporal resolution problems. Endoscope technique is another way to ascertain the disease sites. These endoscopes make patients suffering painful examination process. Radioactive imaging methods provide sensitivities beyond the nanomolar range. However, the high cost as well as radioactive injection limits its application. Therefore, the development of a non-invasive cheap imaging technique is very important.

Near-infrared fluorescence imaging, in particular, provides many advantages as a noninvasive technique for real-time in vivo monitoring of biological information in living subjects without the use of ionizing radiation. Near-infrared fluorescent dyes are one of the commonly used fluorescent imaging probes. These fluorescent dyes greatly decrease the interference brought by the self-absorption of substances and the autofluorescence, increase detection selectivity and sensitivity, and reduce the damage to human body, which are beneficial for bio-assay. These advantages are attributed to the dyes' maximum absorption spectrum of 650~900nm. Cyanine dyes are class of near-infrared fluorescent dyes.

These dyes can effectively avoid the background interference caused by the self-absorption of biological specimen and autofluorescence. Therefore, such dyes are often used as fluorescent labels in the body or made into fluorescent probes. Among the cyanine dyes, indole heptamethine cyanine dyes are the most frequently reported in the research field. They are composed of indole heterocyclics, heptamethine chains, and N-substituent side chains. Recently, many research works were focused on its application of fluorescent imaging probes, since they will avoid effectively self-absorbance in vivo and autofluorescent background. The research work on the molecular structure alteration or improvement designed for increasing the stability and spectrum properties of indole heptamethine cyanine dyes has always been a concern. With indole heptamethine cyanine dyes as the parent, these novel indocyanines having good fluorescent imaging properties and photostability, can be used in fluorescent imaging analysis as fluorescent labeling reagents.

The contents of this paper are presented as follows. Chapter 1 summarizes the recent research progress of the cyanine dyes, particularly the indole heptamethine cyanine dyes, based on the structures, properties, and biological applications of near-infrared fluorescent probes, as well as literature review. This chapter envisages the development trend of near-infrared fluorescent probes.

Chapter 2, 3 and 4 previous research works are introduced. The research works used indole heptamethine cyanine dye as well as substituted meso-position chlorine atom of the parent and considered photostability and water solubility. This chapter synthesizes three kinds of new near-infrared cyanine dyes. The new dyes create a significant breakthrough in Stoke's shift (a key spectrum property). The indole heptamethine cyanine dyes has better photostability and greater the

Stoke's shift than the ordinary polymethine cyanine dyes. Studies have proven the activity of the three kinds of dyes. They have better fluorescence activity enabling the imaging in cells and relatively small cytotoxicity.

In Chapter 5, describes the development of molecular biology in recent years leading to many new targets in tumor treatment, such as the discovery of the methionine dependence of tumor cells. The present study revealed IR-822-Met, a near-infrared fluorescent probe. The molecular structure of this probe is proven by $^1\text{H-NMR}$ and EI-MS, with the methionine dependence of tumor cells, through the covalent bonding of linker groups and methodize (a small-molecule compound having certain dependence) based on the molecular structure of IR-820 (a near-infrared fluorescent dye). The optical properties of the fluorescent probe and the imaging process of tumor-bearing mice were analyzed through ultraviolet spectrophotometer, near-infrared fluorescence spectrometer, and near-infrared fluorescence imaging system. The results show that the probe IR-822-Met had a targeting effect. The compound is expected to provide a new idea for the future diagnosis of tumors.

In Chapter 6, we have described the synthesis of near-infrared (NIR) fluorochrome IR-822-labeled 2-amino-2-deoxy-D-glucose (DG) for optical imaging of tumors in mice. NIR fluorescent dye IR-820 was subsequently conjugated with 3-Mercaptopropionic acid and 2-amino-2-deoxy-D-glucose to form IR-822-DG. The cell experiments and acute toxicity studies demonstrated the low toxicity of IR-822-DG to normal cells/tissues. The dynamic behavior and targeting ability of IR-822-DG in normal mice was investigated with a NIR fluorescence imaging system. The *in vitro* and *in vivo* tumor targeting capabilities of IR-822-DG were evaluated in tumor cells and tumor bearing mice, respectively. Results

demonstrated that IR-822-DG actively and efficiently accumulated at the site of the tumor. The probe also exhibited good photostability and excellent cell membrane permeability. The study indicates the broad applicability of IR-822-DG for tumors diagnosis, especially in the glucose-related pathologies.

In Chapter 7, composed of a superoxide anion ($O_2^{\bullet-}$) responsive group and an H^+ responsive group, a newly synthesized reaction-based near-infrared (NIR) imaging probe (IR-747-SAPH) has been shown to feature high selectivity toward HepG2 cell $O_2^{\bullet-}$ and H^+ fluxes. Fluorescence intensity of IR-747-SAPH responds proportionally to changes in $O_2^{\bullet-}$ and H^+ concentrations. IR-747-SAPH was proven sensitive toward H^+ changes and presents high photostability. Favorable features of this probe also include convenient cell loading, easy staining of cells, and excellent biocompatibility. Most importantly, the probe provides reliable NIR fluorescent signal to changes in $O_2^{\bullet-}$ and H^+ levels in HepG2 cells.

This book was completed under the kind care and careful guidance of my tutor, professor Ji Min. Professor Du Wen and all the teachers and students in the laboratory gave me careful guidance and help in the experiment and the writing of this book.

Limited to the level of the author, the book is inevitably wrong and inappropriate, please readers criticism.

Chunlong Sun

20 March, 2019

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1 Advances in the Biological Application of Indoles Heptamethine Cyanine Dyes Near-infrared Fluorescent Probes

1.1 Introduction

Indocyanine dyes are the most widely used class of cyanine dyes, its spectrum is located in the near infrared region of 650~900nm^[1]. According to the literature, indocyanine dye is the main fluorescent dye used to detect protein, hydrogen ion concentration, metal ion and tumor cells in vivo^[2]. Indoles hemocyanin dyes are composed of indoles heterocyclic rings, hemocyanine chains and n-substituted side chains, and their structures are shown in Fig. 1.1^[3]. In recent years, Indocyanine dyes are increasingly used in biology, by introducing active groups into indoles hemocyanine

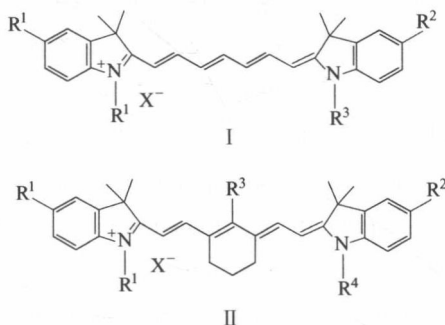


Fig. 1.1 Indocyanine dye structure diagram

R¹, R² = H, CH₃, SO₃, etc; R³, R⁴ = alkyl, aryl, etc;

X = Cl, Br, I, ClO₄, etc

dyes or changing their structural nuclei, the newly generated fluorescent probes have different biological functions, which has become a research focus of fluorescence imaging technology. In this section, the application of indoles heptamethine cyanine dyes in bioassay was reviewed from the aspects of pH changes in vivo or in vitro, detection of trace metal ions and active small molecules, and targeted imaging of tumor cells, so as to provide reference for the further development of fluorescent probes for biological science.

1.2 pH Application of Sensitive Near-infrared Fluorescent Probe

Organisms have the ability to regulate the acid-base balance in vivo. However, if the organism only produces malignant diseases, the organism's ability to regulate the acid-base balance will be weakened, leading to the imbalance of the organism's acid-base balance and the pH value of body fluids exceeding the normal range^[4-6]. According to the literature, some dyes show different absorption or fluorescence properties with the change of pH value^[7-12]. This pH measurement method, which shows different optical signals with the change of pH, can make up for the deficiency of other methods for detecting pH^[13,14]. Be reported in the literature, fluorescence pH method is characterized by high detection sensitivity, simple operation and convenient use^[15]. Fluorescence pH method is not only convenient for fluorescence biomolecular study, but also can detect the pH change in the cell in real time. The pH fluorescence probes can be divided into pH fluorescence probes for detecting the nearly neutral range and pH fluorescence probes for detecting the acidic environment. The intracellular acidity is about: 4.5~6.0, the pH range of cytoplasm is about 6.8~7.4. Many normal physiological processes in cells and organelles are associated with intracellular pH values^[16,17]. It has been reported in the literature, changes in intracellular pH are associated with muscle spasm, cell proliferation and ap-

optosis, ion transport and homeostasis, multidrug resistance, malignancies, endocytosis, and alzheimer's disease^[18]. Therefore, hydrogen ion change is one of the main research targets in vivo, and pH sensitive near-infrared fluorescence probe provides a new method for effectively monitoring pH change in vivo.

Ap-cy, a new near-infrared pH fluorescent probe, was designed and synthesized by introducing 3-aminophenol into the parent nucleus of heptamethine cyanine dye. The light stability experiment shows that the probe Ap-cy has good light stability. Phagocytic experiments showed that the probe had good cell membrane penetration and strong intracellular pH sensitivity. The results of pH experiment showed that the fluorescence intensity of the probe increased exponentially in the range of 4.0~6.5 pH. Under alkaline conditions, the pH sensitive range is 10.5~11.8. Fluorescence imaging of HepG2 cells showed that the probe could monitor the change of hydrogen ion concentration in living cells^[19].

Tang Bo experimental group^[20] designed and synthesized a new pH-sensitive probe 1. The probe can be used to monitor the slight fluctuation of pH value. The probe itself had a pH of 7.1 and showed a strong sensitivity with the change of hydrogen ion concentration in the pH range of 6.70~7.90. Experimental results show that the probe can perform real-time, in situ fluorescence imaging in living cells, effectively avoid interference of biological tissue and spontaneous fluorescence, and has important biological application value. In the same year, Kiyose K et al.^[21] also reported a near-infrared pH fluorescence probe based on FRET mechanism. The structure of Cy-19 is shown in Fig. 1.2 below, when the probe is combined with H⁺, the absorption peak and emission peak of the heptamethine cyanine dye have red shift, and the absorption spectrum after red shift almost has no overlap with the emission spectrum of pentacyanine dye, and the fluorescence of pentacyanine dye is emitted.