Near-infrared fluorescence probe based on selective recognition of glutathione

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Abstract: Glutathione (GSH) is an important bioactive molecule, and its content is closely related to the stability and health of the intracellular environment. Therefore, the detection of GSH is of great significance for life science research. Near-infrared fluorescence probe can effectively avoid fluorescence quenching and enhance fluorescence signal, so it is often used in the detection of GSH. In recent years, the development of near-infrared fluorescence probes has been rapid, and several series of near-infrared fluorescence probes have been reported for the detection of GSH. Based on this, a new type of NIR fluorescence probe was designed and synthesized in this paper. Through its recognition with glutathione (GSH), the fluorescence of the probe was enhanced after reaction with glutathione. Because the probe has selective recognition of GSH, high sensitivity and certain biocompatibility, it can be used for the detection of GSH content in vivo.

Key words: selective recognition; Glutathione; And near-infrared fluorescent probes

Introduction

Glutathione (GSH) is the most important functional amino acid in vivo, and its bioactivity is closely related to its content, so it is of great significance to develop fluorescent probes targeting GSH. In this paper, a novel near-infrared fluorescence probe based on the reaction of GSH with different groups to produce fluorophore is introduced. The probe can selectively recognize GSH and perform fluorescence detection. The experimental results show that the probe has high selectivity and sensitivity, and has a good application prospect, which can provide ideas for the design of glutathione fluorescence probe.

1. Research background

Glutathione (GSH) is an important non-enzymatic antioxidant in vivo. It can combine with a variety of proteins to form complexes in cells, participate in multiple signal transduction pathways, and has a wide range of biological functions. GSH plays an important role in many physiological processes in vivo, including anti-oxidation, anti-inflammation, anti-apoptosis, etc. Therefore, the development of fluorescent probes targeting GSH is of great significance. Near-infrared (NIL-F) fluorescent probe is a new kind of probe with special optical properties, which can be used to detect the content of GSH in biological systems. Near-infrared (NIL-F) fluorescent probes are of great significance in biological analysis and medical imaging, but the current NIR-F-based near-infrared fluorescent probes mainly use N atom as the recognition site. In addition, there are two main problems with NIR fluorescent probes:

First, due to the strong absorption peak of the N atom, the excitation wavelength of the NIR fluorescence probe is usually much shorter than that of the NIR spectrum, which leads to the excitation wavelength of the probe is usually shorter than that of the NIR spectrum.

Second, the near-infrared fluorescence probe is susceptible to interference by various factors in the detection process. In order to solve the above problems, in recent years, many scholars have carried out studies on NIR fluorescence probes based on N atom as the recognition site.

2. The concept and characteristics of NIR fluorescent probes

Near-infrared (NIR,645~780 nm) fluorescent molecular probes refer to a class of near-infrared fluorescent molecular probes with high fluorescence quantum yield and short excitation and emission wavelengths. Its biggest feature is that both excitation and emission light are in near-infrared bands, so it has high cell and tissue penetration, and can be detected in biological bodies. Nir fluorescent probes have the following characteristics:

First, the excitation wavelength of the NIR fluorescent molecular probe is short, so the absorption of the detected substance is relatively less, and the fluorescence intensity is high;

Second, the emission wavelength of the NIR fluorescent molecular probe is short, which will not interfere with the biological function of the detected substance itself, and can achieve high detection sensitivity in the biological body;

Third, the NIR fluorescent molecular probe has good biocompatibility and stability;

Fourthly, NIR fluorescent molecular probes can be used for real-time monitoring of various factors in biological systems.

In recent years, due to the application of photoconversion materials and biocompatible materials in fluorescence probes, NIR fluorescence molecular probes have a good application prospect. In addition, photoconversion materials and biocompatible materials also provide a basis for the development of new NIR fluorescence probes.

3. Overview of instructions

The invention relates to a near-infrared fluorescent probe for selective identification of glutathione embedded with dinnaphthalene as raw material, in which a crude solution of amino fossil merene quantum dots is obtained through nitric acid reflux and hydrothermal reaction, and then a solid amino fossil merene quantum dot is obtained through filtration, dialysis and freeze drying. The amino fossil merene quantum dots react with p-cyanobenzoic acid in the presence of EDC and NHS, and then avoid photodialysis and freeze drying, and finally obtain a composite material named p-cyanobenzoic acid-graphene quantum dots. The composite exhibits excellent photoelectric

properties, low toxicity and biocompatibility, making it an excellent fluorescent probe in vivo. When glutathione is added, the sulfhydryl group on glutathione attacks the cyanide group in the composite nanomaterial and produces fluorescence quenching, so that glutathione can be fluorescence detected at close range.

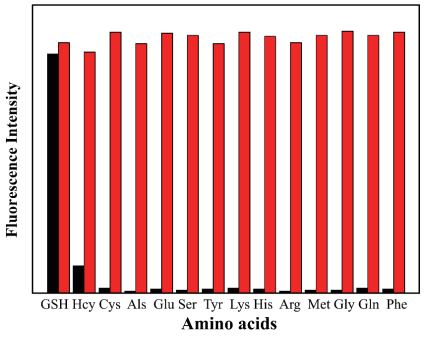

Figure 1. Selective recognition test

4. Near infrared fluorescent probe for selective detection of glutathione

(1) Technical field

The invention mainly focuses on the technical field of preparing fluorescent substances, in particular relates to a near infrared fluorescent probe for selective detection of glutathione.

(2) Basic technology

When detecting GSH content, nano-fluorescent probes are often interfered by other thiols in the body, especially Hcy and Cys, which cannot ensure the high precision of GSH identification. At the same time, most near-infrared fluorescent probes have high biological toxicity. In addition, the large surface area, stable physicochemical properties, good cell permeability and easy biodegradability of graphene quantum dots (GQDs) make them excellent drug carriers for visualization in real-time monitoring systems, without the use of external dyes, and regardless of the inherent fluorescence of GQDs. At the same time, due to their unique structure, GQDs can improve the ability to specifically identify tumor cells. In addition, due to its large specific surface area, biocompatibility and ease of modification, as well as its fluorescence properties due to its unique size effect, graphene quantum dots have potential applications in the early diagnosis and imaging of tumors and are promising for future development.

(3) Content of invention

The specific preparation method mainly includes the following steps:

(1) The reserve solution of amino fossil ink quantum dots was obtained from the added diaminaphthalene by reflux of nitric acid and hydrothermal reaction, and then the reserve solution was filtered, dialysis and freeze-drying to obtain solid amino fossil ink quantum dots;

(2) In the presence of 1-(3-dimethylaminopropyl) -3-ethylcarbodiimide (EDC) hydrochloride and n-hydroxysuccinimide (NHS), the amino fossil merylene quantum dots were reacted with p-cyanobenzoic acid for 20 minutes, followed by photoprotective dialysis for 2 days, and finally freeze-dried to obtain p-cyanobenzoate-graphene quantum dot composites. The composite was used as a near-infrared fluorescent probe to identify glutathione.

At 80℃, the above phenonaphthalene and nitric acid reflux for 12 hours, can be dissolved in a yellowish solid, then dissolved in N, n-dimethylformamide, and at 200℃ to 100mL para-polyphenol reactor hydrothermal reaction for 5 hours.

Next, the amino fossil ink quantum dot stock solution is filtered with a 0.22 micron filter, and then the obtained filtrate is placed in a dialysis bag and dialysis is performed for 2 days at a dark place of 100-500 Da.

After that, the mass ratio of aminofossil merene quantum dots to p-cyanobenzoic acid was 1:3-8.

According to the preparation method, the invention also provides a near infrared fluorescent probe for preparing glutathione recognition.

Beneficial effect: The graphene quantum dot selected by the invention has the excellent characteristics of both graphene and quantum dot at the same time, excellent photoelectric performance, low toxicity, good biocompatibility, and is an excellent living fluorescent probe. The invention can effectively change the electron density and adjust the band gap through N doping and NPS coupling, so that the fluorescence emission spectrum is changed and the emission is closer and closer to the near infrared region. The p-cyanobenzoic acid selected in the invention not only increases the size of the graphene quantum dot, but also adjusts the band gap of the p-cyanobenzoic acid as the electronic group, and because it is a six-membered ring structure, the fluorescence emission spectrum of GQDs can be redshifted through the size effect, so that p-cyanobenzoic acid and p-cyanobenzoic acid can be used to modify the fluorescence emission spectrum of the graphene quantum dot. In this way, the p-cyanobenzoic acid and graphene quantum dot composite material can be successfully used as a fluorescent probe. The fluorescence probe showed good selectivity for glutathione.

(4) Specific implementation details

Embodiment 1: Figure 2 shows how a fluorescent probe in the near infrared region is made, as follows:

1) A yellowish 1,3, 6-trinitropyrene was obtained by heating 2 grams of diamonaphthalene with 160 ml of nitric acid at 80 ° C for reflux for 12 hours; 1,3, 6-trinitropyrene was washed with DMF, dried in an oven at 60℃ and stored as a solid, mixed with 1.2M ammonia and ultrasonic treatment for 2 hours, and hydrothermal reaction was performed in a 100 ml para-phenol reactor at 200℃ for 5 hours;

2) the mixture obtained from the hydrothermal reaction was filtered through a 0.22 micronic filter to remove the excessive substance, and was dialysis in a 100-500Da dialysis bag for 2 days. Finally, the solution in the dialysis bag was freeze-dried to obtain solid graphene quantum dots, which were stored in a refrigerator at 4°C and named NH_2-GQDs.

3)NH_2-GQDs was prepared into 5 mg/mL methanol solution and reacted with 5 mg/mL p-cyanobenzoic acid in the presence of 15 mM 1-(3-dimethylaminopropyl) -3-ethylcarbodiimide hydrochloride and n-hydroxysuccinimide for 20 minutes. After that, the p-cyanobenzoic acid-graphene quantum dot composite material was obtained by freeze-drying in 100-500 Da dialysis bag, which was protected from light for 2 days, and named NH_2-GQDs-CN-1.

FIG. 3 (a, c, d) and 2b show TEM and particle size distribution of NH_2-GQDs at different magnifications, respectively. As shown in the figure, the amino fossil ink quantum dots prepared by the experiment are uniform in size, and the diameter is basically concentrated in 3 nm, and the maximum diameter is not more than 5 nm.

Figure 4 shows the infrared absorption spectra of NH_2-GQDs, 4-cyanobenzoic acid and NH_2-GQDS-CN-1 composites. As shown in the figure, the characteristic absorption peaks of NH_2-GQDS-CN, such as N-H and CO-NH bonds, are obvious, which proves that NH_2-GQDs and p-cyanobenzoic acid are connected by relatively safe chemical bonds. In short, the preparation of NH_2-GQDS-CN composite has achieved a certain degree of success.

FIG. 5a shows the ultraviolet absorption spectra of NH_2-GQDs. As can be seen from the figure, the UV-visible absorption spectrum shows a characteristic peak at 360 nanometers, which is attributed to the n→π* transition of C=O. FIG. 4b shows the fluorescence spectra of NH_2-GQDS-CN-1 at different excitation wavelengths (360, 370, 380, 400, 410, 420, 440, 460, 500 and 540 nm). It can be seen from the figure that the maximum excitation wavelength of NH_2-GQDs is 460 nm, and the emission wavelength is 550 nm. Under 365nm UV lamp, amino-fossil merene quantum dots show bright yellow fluorescence, indicating that it is a quantum dot with good emission characteristics and high fluorescence quantum yield.

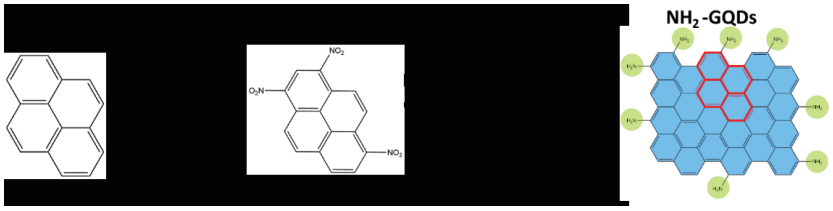

FIG. 2 Schematic diagram of the preparation process of amino GQDs

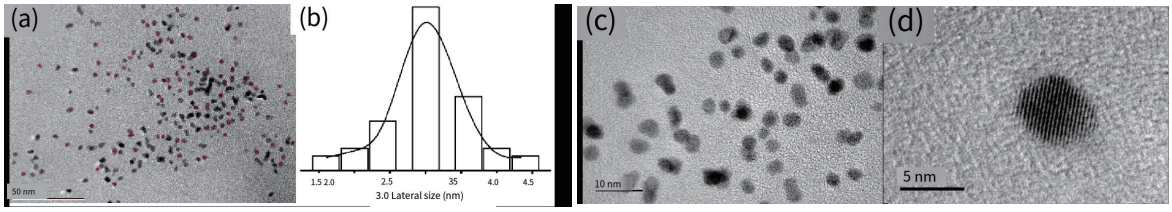

FIG. 3 TEM images of amino GQDS at different magnifications (a, c, d); b particle size distribution map

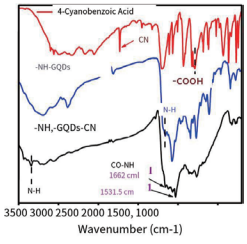

Figure 4 Infrared absorption curve

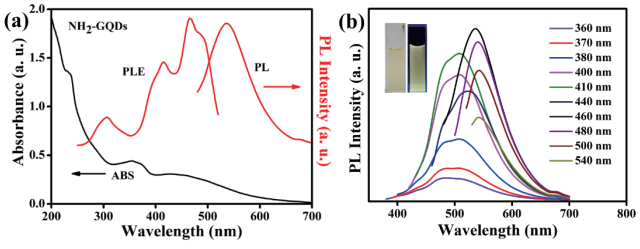

FIG. 5 Optical properties of a amino GQDs

FIG. b Fluorescence curves at different excitation wavelengths

Epilogue

Although embodiments of the invention are described above, the invention is not limited to the uses described in the specification and embodiments, because it can be applied in a variety of fields as long as it is suitable for the invention. It may be further modified in other respects for persons more familiar with the field, and the invention is not limited to corresponding requirements and descriptions, nor to specific details, as long as it does not deviate from the general concepts defined in the corresponding field of application.

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