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研究課題名（和文）Precisely activating ferroptosis by Fe(II)-triggered drug release for enhanced cancer therapy

研究課題名（英文）Precisely activating ferroptosis by Fe(II)-triggered drug release for enhanced cancer therapy

研究代表者

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研究成果の概要（和文）：Fe2+応答性オキシムエステルの開発は、主に切断時の蛍光変化を評価することにより行われた。さまざまな置換基を導入した蛍光色素を合成した。電子特性が反応に与える影響も評価された。蛍光変化によるFe2+切断の反応性を確認するために、ターンオン型と比率型の分子を設計した。次に、フェロトーシス誘導プロドラッグをオキシムエステルに導入し、薬物放出と蛍光出力を評価する。最終的に、最適化された分子を培養細胞とマウスモデルに適用します。さらに、酸性腫瘍微小環境の検出のために、pH応答性の環状部分構造を持つシアニン色素も設計した。そのpH応答性、水溶性、活性化凝集の効果により、迅速な腫瘍検出することが成功した。

研究成果の学術的意義や社会的意義
癌症は常に人類の健康を脅かしています。近年の癌死亡率の増加に伴い、癌で亡くなる人の割合が増加しています。フェロプトーシスは、鉄過剰と脂質過酸化によって新たに定義された細胞死の一つであり、がん治療に大きな潜在性を持っています。鉄触媒による結合切断有機反応は、化学生物学における重要なツールを提供します。この研究は、がんプロドラッグのフェロプトーシス誘導剤としての開発のために、新しいFe2+トリガーでオキシムエステルの切断に挑戦しています。これにより、標的リガンドと鉄依存性の薬物放出機構によって、がん細胞でフェロプトーシス誘導剤を選択的に放出する可能性があり、生体内の分子イメージングも実現します。

研究成果の概要（英文）：The development of a Fe2+-responsive oxime ester was conducted by evaluating fluorescent changes upon cleavage. Fluorescent dyes such as coumarin, rhodamine, and cyanine containing oxime ester moieties were synthesized with various substituents. The effects of electron properties on reaction efficiency were evaluated. To confirm the sensitivity and reactivity of the Fe2+-activated cleavage reaction, turn-on and ratiometric fluorescent molecules were designed and investigated. A ferroptosis-inducing prodrug will be introduced into the oxime ester to assess drug release and fluorescent output. Finally, the optimized molecule will be applied to cultured cells and mice models.
Additionally, we prepared a near-infrared cyanine dye with a pH-sensitive cyclic substructure for detecting the acidic tumor microenvironment. Its pH-responsiveness, water-solubility, and activated aggregation enable rapid tumor diagnosis and clearance from the living system.

研究分野：有機化学、生体分子化学およびその関連分野

キーワード：fluorescence　cancer therapy　diagnosis　iron　ferroptosis　cleavage

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1. Background of the research beginning (研究開始当初の背景)

Ferroptosis is one of newly discovered regulated cell death pathway which tightly relates to biological Fe^{2+} fluctuation. However, studies of ferroptosis are still in infancy, some mechanisms of ferroptosis have not been clarified yet, particularly in regard to contribution of iron load. In recent years, transition metal mediated bioorthogonal chemistry is extremely useful to unravel and manipulate biological processes. The applicant's research group developed a Fe^{2+}-catalyzed oxime ester cleavage reaction which gave a high conversion yield in room temperature.[1] The applicant envisioned that is the iron-mediated metallic catalyst reaction applicable to drug release with the aim of precisely controlling ferroptosis.

2. Research purpose (研究の目的)

By means of novel Fe^{2+}-triggered bond cleavage reaction, the applicant aimed to developing of a new strategy for ferroptosis-based cancer therapy through a controlled drug release (Figure 1). The cancer prodrugs will be released as an active ferroptosis inducer in cancer cells in a circulated manner. Furthermore, the modulated ferroptosis accompanied with fluorescent output in cancer cells allows visualization of fluctuation of cellular iron which is valuable for studying the metabolism of concentrated iron.

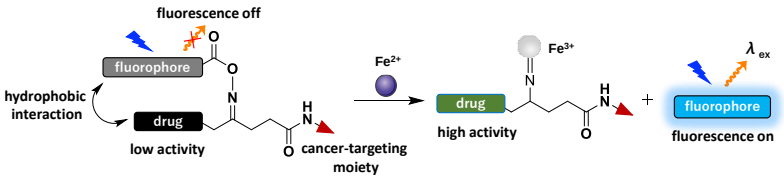

Figure 1. Ferroptosis therapy based on the Fe^{2+}-activatable drug release with fluorescent output.

3. Research methods (研究の方法)

The development and optimization of Fe^{2+}-responsive oxime ester linker in vitro was mainly conducted by evaluation of fluorescent change upon cleavage. The research started the organic synthesis of model molecules. Then fluorescent dyes, such as coumarin, rhodamine and cyanine dye, containing oxime ester moiety were synthesized by introducing various substituents. The molecular design was conducted by the dynamic functional theory (DFT). The effects of electron properties on reaction efficiency also were evaluated. Their Fe^{2+}-responsiveness and time-dependent reactivity were investigated. To confirm the sensitivity and reactivity of Fe^{2+}-activated cleavage reaction by fluorescent changes, the turn-on type and ratiometric type of organic molecules were designed and investigated. Next, the ferroptosis-inducing prodrug will be introduced to check its drug releasing and fluorescence turn-on efficiency. After that, the tumor-targeting moiety like cRGD will be cooperated to achieve its cancer selectivity. Finally, the optimized molecule would next be applied to molecular imaging and tumor-inhibition assays in the cultured cells, living tissues, and tumor-bearing mice models.

4. Research results(研究成果)

As the Fe^{2+}-catalyst oxime ester cleavage reaction was mainly carried out in organic solvents, such as toluene, benzene, which is not suitable for biological applications. To evaluated the reactivity and stability of oxime ester towards Fe^{2+}, we firstly prepared a model molecule **1** and investigate its properties (Figure 2A). As we expected, the molecule **1** exhibited high stability. It kept intact in aqueous solution even elongate time to 24 h. The cleavage reaction by Fe^{2+} was further evaluated by addition of iron salt. Reaction product was confirmed by gas chromatograph- and electrospray ionization (ESI)-mass spectrometry. However, the cleavage product from compound **1** is non-fluorescent emission and small molecule which is hard to be purified and separated. Therefore, a various of fluorescent molecules **1a-1d** based on coumarin dye was developed. Their photophysical properties were investigated by absorbance and fluorescence spectra. Compound **1a** gave a short absorbance (around 340 nm) and fluorescent peak at 412 nm which showed potential toxicity of photo-irradiation (Figure 2B and 2C). In contrast to **1a**, the introduction of diethylamino group at compound **1b** shifted its absorbance peak to 420 nm with a strong fluorescence. Furthermore, the reactivity of **1b** towards Fe^{2+} was

investigated in aqueous solutions. It was found that the cleavage reaction occurred smoothly in a 100% yield. The cleavage product was purified and characterized. However, the photophysical properties of **1b** and product **1b-p** are very similar which made it difficult to distinguish the cleavage by fluorescent change and output. Theory calculation was conducted to predict the electron transfer properties of **1c** and **1d**. An efficient electron transfer would be used to quench the fluorescent intensity.

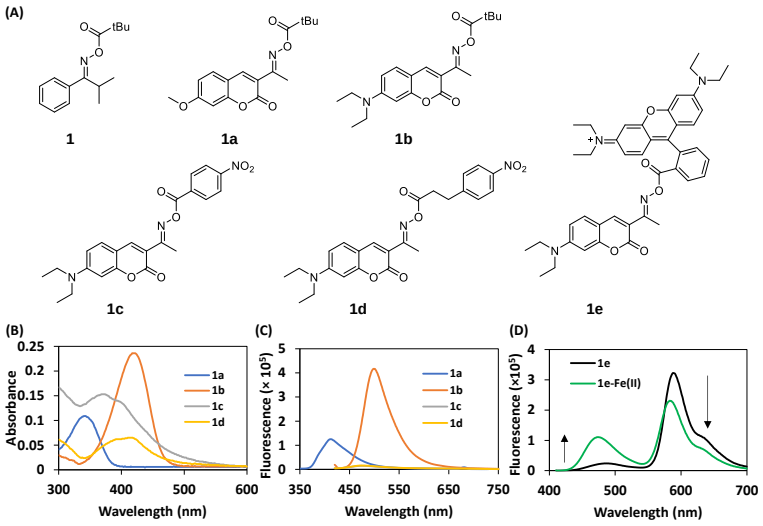

Figure 2. (A) Chemical structures of prepared oxime esters. (B) UV-vis absorbance and (C) fluorescence spectra of **1a-1d** (5 μM) in aqueous solutions. (D) Fluorescence spectra of **1e** in presence and absence of Fe^{2+} in aqueous solutions after 2 h incubation.

It is available to develop the turn-on type fluorescent sensor. The cleavage of oxime ester of **1c** and **1d** was confirmed as expected. Unfortunately, the molecules containing strong electron-withdrawing moiety show low stability and suffer from decomposition. We also prepared a ratiometric type molecule 1e by using the oxime ester to link coumarin and rhodamine. An obvious ratiometric signal change was observed upon the treatment of Fe^{2+}, suggesting a cleavage process occurred along with an increase emission from coumarin at 474 nm (Figure 2D). Next, the prodrug and tumor-targeting moieties will be introduced to evaluate its drug release and tumor-inhibition efficiency, and so on.

Besides, based on the previous research of the applicant, they developed a fluorescent sensor, **CypH2S**, with a pH-sensitive cyclic substructure for detection of acidic tumor microenvironment.[2] Upon pH variation, the sensor underwent structural changes: the closed-ring form, **CypH2S-C**, exhibited suppressed dye aggregation, while the open-ring form, **CypH2S-O**, tended to aggregate in aqueous solutions under slightly acidic conditions (Figure 3A). The pH-responsive aggregation was confirmed by Transmission electron microscopy (TEM) images (Figure 3B). Importantly, **CypH2S** facilitated rapid and selective tumor visualization in a living mouse model via fluorescence imaging of its pH-responsive aggregation with a 1.9-fold tumor/background ratio (< 1 h, Figure 3C). Furthermore, prompt clearance of **CypH2S** from the bloodstream post-tumor detection occurred because of the relatively small size of the aggregates and cell impermeability. These properties would make **CypH2S** a promising candidate for clinical tumor diagnosis, which also tightly relate to and efficient support the

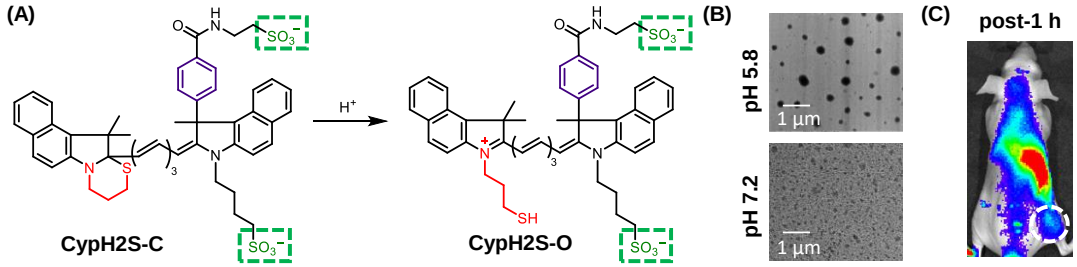

Figure 3. (A) pH-Responsive probe **CypH2S** in an equilibrium between **CypH2S-C** (closed-ring form) and **CypH2S-O** (open-ring form). (B) TEM image of self-assemblies of **CypH2S**. (C) In vivo fluorescence imaging of tumor-bearing mice administrated with **CypH2S** (100 μM).

applicant's current research.

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掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D3TB02956E	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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〔図書〕　計0件

〔出願〕　計2件

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特許、2023-146159	2023年	国内

産業財産権の名称	発明者	権利者
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特許、PCT/JP2023/47011	2023年	外国

〔取得〕　計0件

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関