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Drug-delivery and multifunction possibilities of hypocrellin photosensitizers

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Photodynamic therapy (PDT) has been a routine treatment of tumors and some microvascular diseases, but clinically available photosensitizers are still scarce. Among all kinds of photosensitizers, hypocrellins possess the most characteristics of ideal photosensitizers, such as, high photo-activity but low dark toxicity, fast clearance from tissues. This review is focused on two main topics, drug-delivery problem of hypocrellins and how the environment-sensitive fluorescence of hypocrellins was used for recognition of various biomolecules. Drug-delivery of hypocrellins was mainly achieved in two strategies — preparing the drug-delivery vehicles and finding quantitatively amphiphilic derivatives. Hypocrellin fluorescence originated from the intramolecular proton transfer is very distinct from other kinds of photosensitizers. Recently, it was proved that quantitative hypocrellin fluorescence could not only recognize various biomolecules, including proteins, polysaccharides and lipids, but also distinguish the specific binding from nonspecific binding with some kind of biomolecules. Meantime, hypocrellin fluorescence was pH-sensitive. It is known that tumor cells or tissues have the features of a large amount of lipid, neonatal collagen, over-expression of polysaccharides, and lower pH values compared to normal tissues. According to the relative but not absolute specificity, further studies on quantitative recognition of various biomolecules at a cellular level, may find a new clue to treat tumors by joint usage of photodynamic diagnosis (PDD) and PDT.

Keywords: Hypocrellins; photodynamic therapy; drug delivery; biomolecular recognition.

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1. Introduction

Photodynamic therapy (PDT) has become a regular methodology to treat various tumors clinically,[1] and recently, it has been successfully used to treat some microvascular diseases, such as port wine stains (PWS), age-related macular degeneration (AMD).[2,3] It is known that light, photosensitizer and oxygen are the three primary factors for PDT but photosensitizer is the key important one. An ideal photosensitizer should possess high PDT activity but low dark toxicity as well as fast clearance from tissues, in addition, it should be readily delivered but not seriously sacrifice the PDT activity *in vivo*. Besides the photosensitization activity to produce reactive oxygen species, the photosensitizers are fluorescent. It has long been hoped that a photosensitizer might be used for photodynamic diagnosis (PDD) and PDT at the same time,[4] which may be a good strategy to conquer cancers, however, lack of specific target and fluorescence signal prohibit the program.

Hypocrellins, including hypocrellin A (HA) and hypocrellin B (HB) (shown in Fig. 1), exhibit most of the characteristics of ideal photosensitizers mentioned above.[5–7] However, their low absorption in the phototherapeutic window (600–900 nm) makes them not suitable for PDT of solid tumors.[8] In addition, hypocrellins are lipophilic organic compounds, which promotes the cellular uptake[9] but prohibits the drug-delivery and bioavailability.[10] Besides PDD depends on the typical fluorescent signals of a photosensitizer on the targets. It was indicated that some biological molecules were over expressed in tumor cells or tissues,[11,12] however, they are quantitatively but not qualitatively specific relative to normal cells. Therefore, tumor cells or tissues may not be recognized by non-specific fluorescent information. It was known that hypocrellin fluorescence was originated from intramolecular proton transfer[13] and sensitive to solvents.[14] In this review, some recent progresses of researches on the drug-delivery and environment-sensitive fluorescence of hypocrellins are concerned.

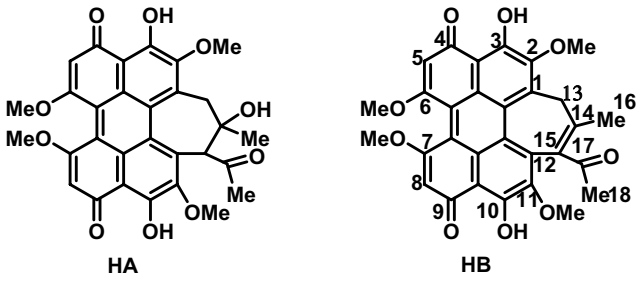


Fig. 1. The chemical structures of HA and HB.

2. Drug-Delivery of Hypocrellins

2.1. *Drug-delivery vehicles*

One important strategy for clinical application of lipophilic photosensitizers is to prepare drug-delivery vehicles in nanometer sizes with biocompatible materials, which are water soluble and readily control drug-delivery *in vivo*.

2.1.1. *Liposomal hypocrellins*

The liposome is composed of lipid bilayers self-assembled naturally or artificially by phospholipids. The liposome has good biocompatibility, targeting, controllable rates of drug efflux, and has been widely employed as vehicles for lipophilic drugs, for instance, verteporfin, a PDT drug for AMD, is clinically used in a liposomal preparation.[15] Ultrasonic method was previously used to prepare the liposome of HA and HB which can retain about 70% of PDT activity of HA or HB.[16,17] A pharmacokinetic study in model animal indicated that the liposomal HA had much higher accumulation in tumor tissues than HA in DMSO, suggesting that the liposome formulation could preferentially target tumor tissues.[18] Photosensitization of liposomal HB produced superoxide anion radicals, hydroxyl radicals and singlet oxygen, suggesting both Types I and II photosensitization mechanisms involved.[16] Because liposome in solution is not stable, solid liposome was prepared by reverse evaporation-lypophilization drying.[19] More recently, liposomal hypocrellin powder was prepared by high pressure homogenization method.[20] This method not only promoted the drug loading up to 1 mg/mL, but also got rid of tween 80 which was not recommended by pharmacopoeias, in addition, the liposome size was distributed uniformly and controllably. Taking chicken combs as models of PWS, it was proved the liposomal hypocrellin B could selectively destruct the dermal microvasculatures, but not hurt overlying epidermis with a HB dose of 0.5–1 mg/kg and a light dose of 120 J/cm^2, suggesting the safety and efficiency.[21]

2.1.2. *Nanomicelles of hypocrellin*

A micelle is prepared by the use of surfactant molecules to load lipophilic drugs. HA and HB triton X-100 micelles were prepared with the particle sizes in about 5 μm.[22–24] It was reported that triton X-100 micelles of HB could maintain most of the photosensitization activity, however, the sizes of micelles are too large to be used as PDT drugs. But this formulation may be used as photodynamic pesticides.

2.1.3. *Nanoemulsion of hypocrellin*

The emulsion is a preparation of heterogeneous system formulated by two or more liquids which are not inter-solvable. Generally, the particle sizes of currently clinically used emulsions are in micrometer level, which is too large to be used as PDT drugs. Recently, the hypocrellin emulsion was prepared in the sizes distributed from 30 to 60 nm[25] with a drug loading of 1 mg/mL, which is suitable for use in PDT. In addition, the PDT activity of the emulsion preparation is the highest among all the preparations. The hypocrellin emulsion is readily prepared with low-cost materials, however, the storage time is no more than six month, which is too short to satisfy the quality standard of a new drug.

2.1.4. *Nanoparticles of hypocrellins with bio-materials*

Nanoparticles are known as nanospheres or nanocapsules, which carry drugs inside or outside particles by physical, chemical adsorption or even covalent combination. Previously, hypocrellin nanoparticles were prepared with gelatins or polysaccharides in the sizes of about 100 nm.[26,27] The preparation is not only drug-delivered but also remains most of the PDT activity. Until now, many kinds of hypocrellin nanoparticles were reported, including HA-Silica nanoparticles, nanoscale porous ceramic nanoparticles, pH-responsive silica nanoparticles, TiO_2 nanoparticles, hierarchical gold/copolymer nanostructures or lipid-coated gold nanocages, etc.[28–33] *In vitro* studies showed most of these formulations remained most of the PDT activity of their parents, but whether they can be used *in vivo* is still questionable. The formulations are usually very stable due to presence of some hardeners, however, controlled-release drugs may be appropriate for PDT of solid tumors, but not for PDT of microvascular diseases.

2.2. *Chemical modifications of hypocrellin structures*

Aqueous solubility is essential for both drug-delivery and bioavailability,[34] but lipophilicity is also necessary for cellular uptake which is closely related to PDT activity *in vivo*. Generally, a lipophilic photosensitizer exhibits high cellular uptake which is necessary for PDT activity *in vivo*, but its self-aggregation may lead to a risk of vascular embolization. On the other hand, a hydrophilic photosensitizer is ready for drug-delivery, but its little cellular uptake leads to low PDT activity. For about 20 years, chemical modifications of hypocrellins were focused on improving the aqueous solubility, however, the improvement had only a relative significance — the solubility of some derivatives was somewhat higher than their parents (several μM level), but it was either too low to satisfy clinical requirement for drug concentration, or too high to have a cellular affinity. Consequently, in a viewpoint of clinical requirement for drug-delivery, an ideal derivative should have a quantitative instead of qualitative amphiphilicity, i.e., the lipophilicity should be kept as large as possible as long as the aqueous solubility satisfies the clinically required concentration of the drug.[34] Generally, clinically required concentration (C) could be estimated according to the following equation[35]:

$$C = \frac{D_{\mathrm{HB}} \times W \times A_{\mathrm{HB}}}{V \times A_{\mathrm{Derivative}}}. \tag{1}$$

In the equation, D_{HB} is 0.5 mg/kg based on the animal experiments for PDT of port wine stains (PWS)[36]; W is an average weight of a person, 80 kg; V is commonly acceptable volume for one time intravenous injection, 30 mL and $A_{\mathrm{HB}}/A_{\mathrm{Derivative}}$ is ratio of PDT activity of HB to the derivative. So if the PDT effect of a hypocrellin derivative is equal to that of HB, the minimal concentration for directly intravenous injection is calculated to be 1.3 mg/mL, which indicates the threshold for the clinically required solubility.

2.2.1. *Hypocrellin-metal ion complexes*

Hypocrellins facilely react with aluminium(III), magnesian(II), zincl(II) or lanthanum(III) ions to

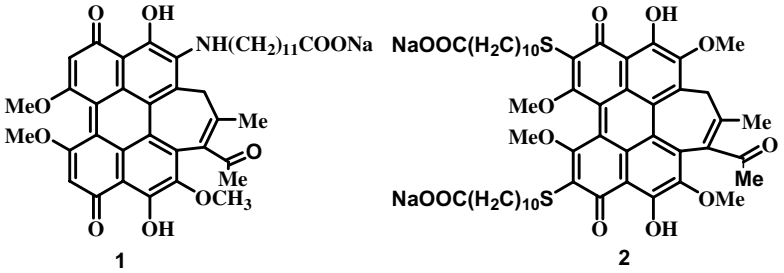


Fig. 2. The molecular structures of **1** and **2**.

form water-soluble complexes. It was reported that hypocrellin-metal ion complexes could enhance both water solubility and absorption in the phototherapeutic window (600–900 nm) of tumors.[37–39] Generally, the molecular weight of the complexes was uncertain perhaps due to formation of polymer-like structures.[37] It was reported that some Cu(II), Co(III) and Oxovanadium(IV) HB complexes with certain molecular weight could result in effective photodamage to DNA than free HB.[40–42] An *in vivo* study indicated the hypocrellins complexes with aluminium ions might be dissociated *in vivo*, then lead to aggregation of free hypocrellins and vascular embolization (unpublished result), which may be a common problem for this kind of complexes.

2.2.2. *Introduction of a polar substituent*

Introduction of a polar substituent to a lipophilic molecule is a common strategy to improve the aqueous solubility. Sulphonate, glycosylate, cyclodextrin and dicysteine substituted hypocrellin derivatives[43–47] were easily soluble in aqueous solution and exhibited some photosensitization activity *in vitro*, but they almost lost the PDT activity *in vivo* due to low cellular uptake.[48] Previously, a theoretical method was developed to evaluate the amphiphilicity of hypocrellin derivatives and a concept of quantitative amphiphilicity was proposed.[49] Evaluated based on this idea, mercapto, amino-acid, ethanolamine, morpholine, piperazine and dipeptide substituted hypocrellin derivatives exhibited higher solubility than their parents,[50–54] but the solubility was lower than the threshold of clinically required concentration as mentioned above. On the other hand, aliphatic amine, aromatic amine or bisamine substituted hypocrellin derivatives were even more lipophilic than their parents.[55–57] It is well known that surfactants are theoretically amphiphilic. However, surfactant-like HB derivatives, sodium 12-2-HB-aminododecanoate and sodium 11, 11′-5,8-HB-dimercaptoundecanoate (**1** and **2** in Fig. 2)[58] exhibited solubility of 0.4 and 3.4 mg/mL, respectively. Evaluated according to the threshold of drug concentration, the former is too low but the latter is high enough for the usage of intravenous injection.

2.2.3. *Quantitative and site-directed modification to optimize the solubility and PDT activity*

Previously, taurine substituted HB (**3**, Fig. 3) was synthesized, its aqueous solubility was more than 9 mg/mL, and the PDT activity was higher than sulfonate substituted HB but far lower than HB,[59] which suggested that prolonging the alkyl line in the substituent may lead to finding the optimized or quantitative amphiphilicity.

Generally, PDT activity of a photosensitizer is mainly dependent on two factors — the cellular uptake and singlet oxygen yield.[60] **3** exhibiting far lower PDT activity than HB is ascribed to lower cellular uptake and the lower singlet oxygen yield. An advantage of hypocrellins is that their structures are easily modifiable by introduction of

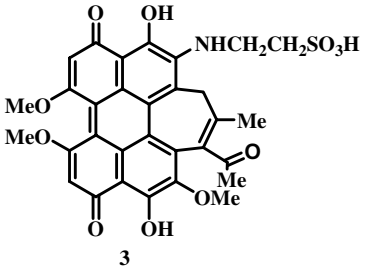
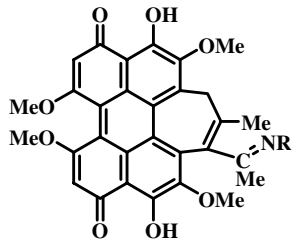


Fig. 3. The molecular structure of compound **3**.



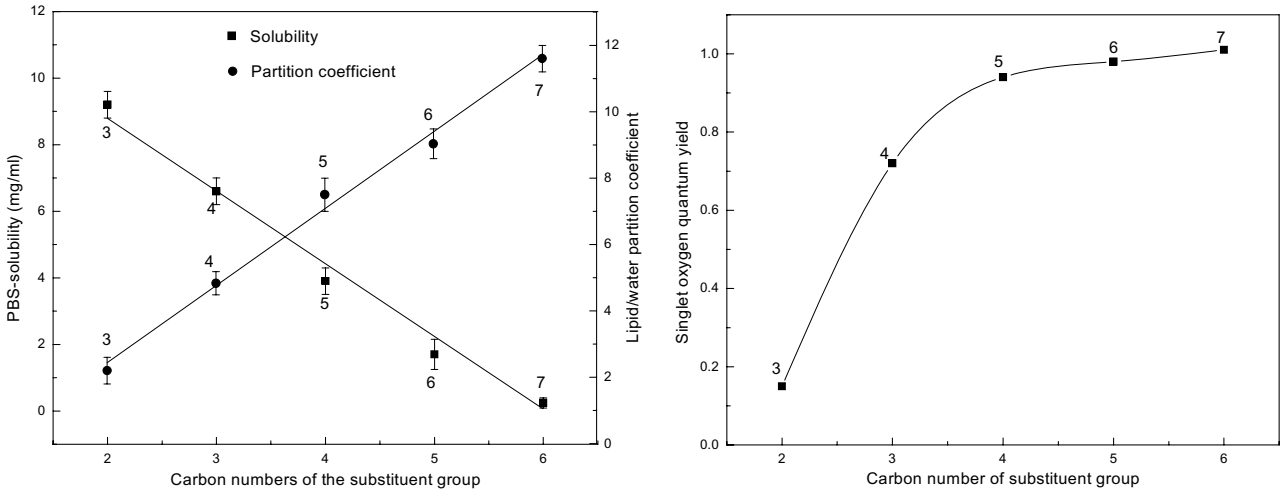
R=$CH_2CH_2CH_2SO_3H$ 4
$CH_2CH_2CH_2CH_2SO_3H$ 5
$CH_2CH_2CH_2CH_2CH_2SO_3H$ 6
$CH_2CH_2CH_2CH_2CH_2CH_2SO_3H$ 7

Fig. 4. The molecular structures of **4**–**7**.

a substituent to site 2, 5, 8, 11, 13, 14 or 17, but the singlet oxygen yields of these derivatives are very different. Generally, substitution to a site on the perylenequinoid ring, such as 2, 5 or 8, leads to lower singlet oxygen yields,[53,61,62] while site-17 substituted derivatives exhibit highest singlet oxygen yields.[63–65] According to these considerations, a series of 17-amino-alkyl-sulfonic acid substituted derivatives of HB with 3, 4, 5 and 6 carbon atoms in the alkyl chain were synthesized (**4**–**7** shown in Fig. 4).[35,64]

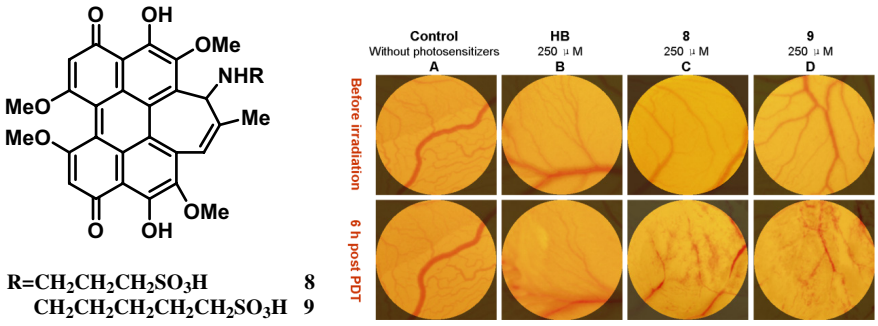
Interestingly, it was found that the aqueous solubility and PC formed linearly decreasing and increasing sequence with increase in the carbon number, in addition, the singlet oxygen yield increased gradually with the carbon atom number, as shown in Fig. 5. Among these derivatives, **6** exhibited the solubility of 1.7 mg/mL, which was just higher than the threshold of required drug concentration, and the singlet oxygen yield as high as 0.98. Consequently, it exhibited much higher PDT activity to human gastric cancer BGC823 cells than the parent HB, with the IC_{50} (defined as the photosensitizer concentration required killing 50% of the cells) of 22 nM compared with that of 40 nM for HB, in addition, it had no dark cellular toxicity.[35] It can be concluded that **6** possesses the optimized amphiphilicity and PDT activity, and can be directly used for intravenous injection without need of preparation of drug-delivery vehicles.

Similarly, 15-deacetyl-13-amino-alkyl-sulfonic acid substituted HB derivatives (**8** and **9**, Fig. 6(a)) were designed and synthesized specially for PDT of AMD.[66] These derivatives with the substituent at the site 13 exhibit the maximum absorption on orange light (580 nm), which may be a proper phototherapeutic window of AMD, because the light not only penetrates tissues no deeper than 1 mm coincided with the diseased targets, but also is less absorbed by visual pigments. Estimated similarly as mentioned above, the solubility of 7.1 or 2.0 mg/mL for **8** or **9** is large enough for intravenous injection, but the latter is better for effective cellular uptake. *In vivo* experiments prove that **9** results in much effective damage to blood vessels than **8** or HB, as shown in Fig. 6(b), with chick chorioallantoic membrane (CAM) as the model.



(a) (b)

Fig. 5. (a) Plot of the PC or the solubility of **3**–**7** to the carbon atom number in the substituent. (b) Plot of the singlet oxygen quantum yield of **3**–**7** to the carbon atom number in the substituent.



(a) (b)

Fig. 6. (a) The molecular structures of **8** and **9**. (b) PDT effects of HB (B), **8** (C) or **9** (D) on the blood vessels of CAM along with that of without photosensitizer as a control (A). First or second line is the blood vessels images of CAM recorded before irradiation or 6 h after PDT.

3. Recognition of Biological Molecules by Environment-Sensitivity of Hypocrellin Fluorescence

In comparison to other kinds of photosensitizers, hypocrellin fluorescence is very sensitive to the microenvironment because it is originated from the intramolecular H-atom transfer process,[13] which means, any microenvironment to strengthen or weaken the intramolecular hydrogen bond will certainly affect the fluorescence. Imaginably, the environment-sensitive fluorescence could be taken as a probe to monitor the microenvironments of biological molecules or tissues. As mentioned above, joint usage of PDD and PDT for treatment of tumors at an early stage may be a right strategy to cure cancers, however, finding a specific differentiation of a tumor cell from a normal one is still a great challenge. For a long time, it was known that tumor cells or tissues possess the characteristics of a large amount of lipid and neonatal collagen, but lower pH values, as well as over expression of biological molecules,[67–69] however, the quantities all being relatively specific but not absolutely specific, make fluorescence diagnosis difficult. According to the quantitative difference of the tumor cells from normal ones, it may be asked whether environment-sensitive fluorescence of hypocrellins can be used to recognize different microenvironments, which may lead to some new clues for early diagnosis of tumors.

3.1. *Solvent-sensitive fluorescence of hypocrellins*

Previously, it was reported that both bulk effect and polarity of solvents had a very pronounced impact on hypocrellin fluorescence, which was ascribed to the particular intramolecular hydrogen bond or intramolecular proton transfer between the keto and enol groups in a hypocrellin molecule.[13,73] It was further confirmed by the methylation of HB eliminating the fluorescence completely.[14] In fact, the fluorescence intensity of porphyrin-like photosensitizers also varied in the solvents with various polarity, but it was completely due to the solvent dependent absorbance.[70] Therefore, the particular fluorescence may be valuable for monitoring the microenvironments of biological molecules or targets.

3.2. *Using hypocrellin fluorescence for recognition of various biological molecules*

The biological molecules on cellular surface include three main kinds including proteins, polysaccharides and lipids. Previously, hypocrellin fluorescent responses to the microenvironments of various biological molecules were investigated and the results were summarized below.

3.2.1. *Specific binding of HB with human serum albumin (HSA)*

HSA is one of the main drug transporters in human blood plasma and contains three homologous domains (labeled I–III) which are divided into two sub-domains (A and B). It was ever reported that hypocrellins could randomly bind to the hydrophobic positions of HSA.[71] Taking HB and the sole tryptophan in sub-domain IIA of HSA as dual fluorescence

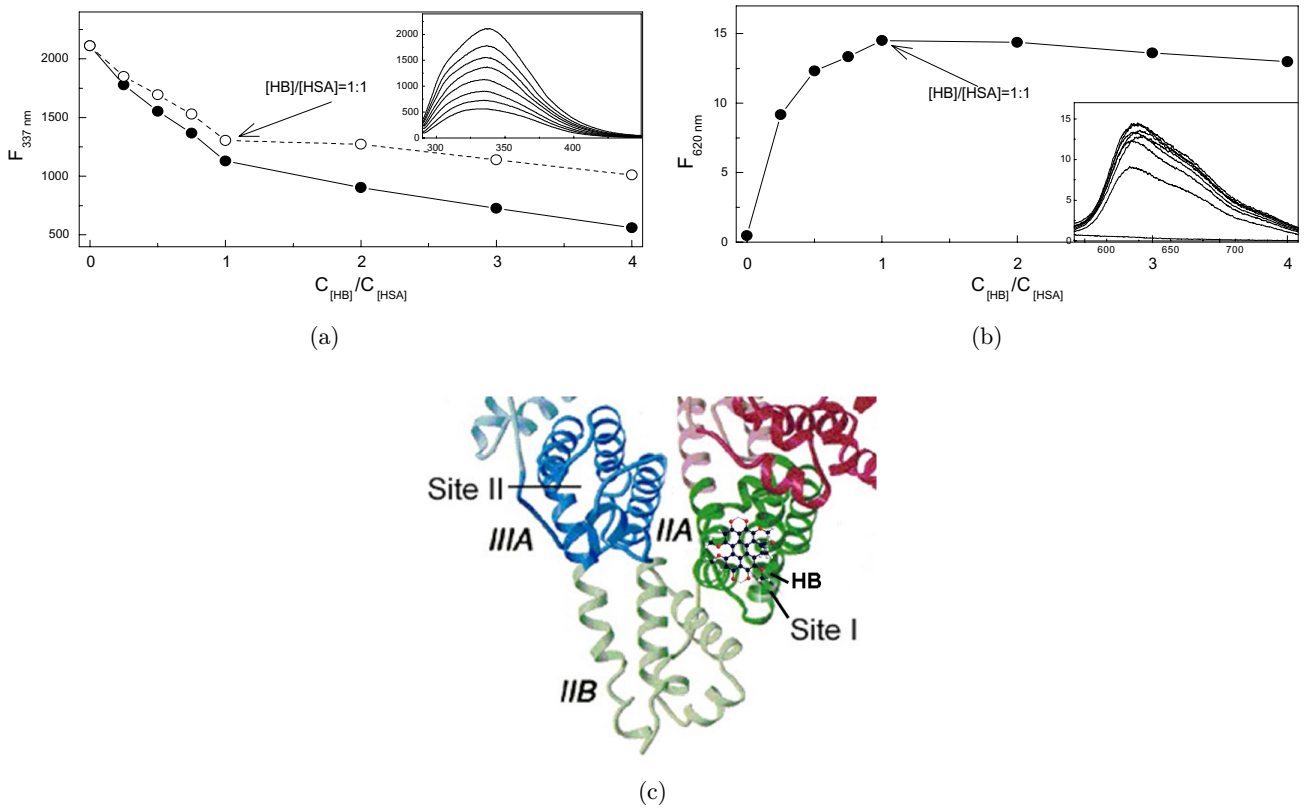


Fig. 7. The plots of the fluorescence intensity of the tryptophan at 337 nm (a) and HB fluorescence at 620 nm (b) to the ratio of concentration of HB to HSA (10 μM) in PBS (pH 7.4). (c) HSA structure and the specific binding site.[72]

probes, it was clarified that HB specifically bound to the site I of HSA with the molar ratio of 1:1, suggested by the common inflection point of the fluorescence intensity, at the concentration ratio of HSA and HB to 1:1, as shown in Fig. 7.[72,73]

3.2.2. *Specific binding of HB to hyaluronan (HYA)*

HYA is a polysaccharide molecule playing an important part in life process, and over-expression in tumor cells or tissues.[74] Previously, the binding and interaction of HB with HYA was investigated by monitoring the spectral responses of HB.[75] Interestingly, with a continuous increase in the concentration of HYA, the absorbance of HB continuously rose until a saturated value, but the fluorescence decreased until quenching completely. Based on the particular fluorescent property of HB and one time increase in the particle size of HYA after interaction with HB, the absorbance increasing relative to that in PBS, was ascribed to binding with the hydrophobic area of HYA, while the fluorescent quenching was ascribed to formation of two intermolecular hydrogen bonds, which completely inhibited the intramolecular proton transfer, as shown in Fig. 8. It was found that both the absorption and fluorescence increased when HB was bound to sodium alginate (SOA), another polysaccharide molecule,[75] suggesting a specific binding of HB to HYA.

3.2.3. *Quantitative fluorescence recognizing various kinds of biological molecules and monitoring binding specificity*

By the use of HSA, bovine serum albumin (BSA) and ovalbumin (OVA) as the models of proteins, HYA and SOA as the models of polysaccharides, and liposome as mimic cell membranes, the spectral responses of HB to the microenvironments in various biological molecules were investigated.[76] According to the absorption and fluorescence spectra, a parameter of $R_{F/A}$, defined as the ratio of the fluorescence to absorbance of HB, was proposed to characterize a microenvironment specifically. Dependence of $R_{F/A}$ values of HB (8 μM) on the normalized biomolecular concentrations or water, benzene solution were shown in Fig. 9. Generally,

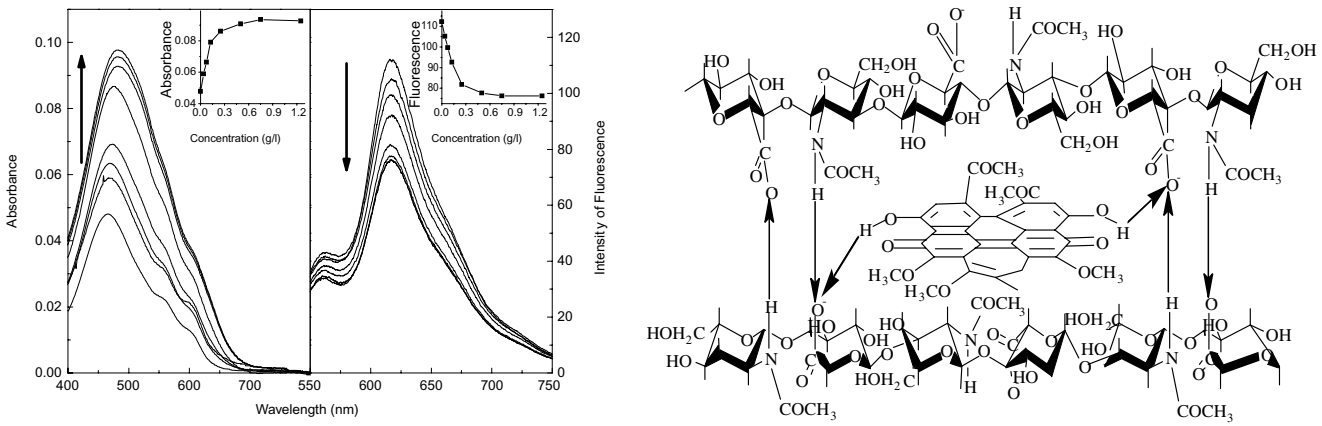


Fig. 8. Absorption (a) and fluorescence spectra (b) of HB (8 μM) in PBS (pH 7.0) in the presence of HYA with a series of concentrations. Insets: The plots of the absorbance and fluorescence intensities to the concentrations of HYA. (c) The structure model of HB binding to two HYA.[75]

the $R_{F/A}$ values of HB form a decreasing sequence in benzene>liposome>protein>water>polysaccharide, which is similar to the sequence of decreasing in the hydrophobicity. For HB in benzene has a perfect hydrophobic environment, it has the highest $R_{F/A}$ value. HB in liposome is completely enclosed by the bilayer phospholipid membranes, but the $R_{F/A}$ value is far lower than in benzene, suggesting that the environment in liposome is not perfectly hydrophobic for presence of some water molecules in semi-fluid liposome.[77] In comparison, the hydrophobic area in the proteins is not completely closed as in liposome, therefore the $R_{F/A}$ values are lower than in liposome. Particularly, hydrophobic area in polysaccharides is half-open, therefore, it has the lowest $R_{F/A}$ value. Although the microenvironment in polysaccharides is more hydrophobic than in water, which should result in increasing of both the absorbance and fluorescence, the $R_{F/A}$ values even lower than in PBS is ascribed to a less increase in fluorescence than in absorbance. The $R_{F/A}$ value of HB in HYA is far lower than that in SOA, due to the specific binding of the former but non-specific binding of the latter. Similarly, as a result of HB specifically binding with HSA, the $R_{F/A}$ value of HB in HSA is far higher than that in BSA and OVA. The $R_{F/A}$ values in BSA and OVA are almost identical, indicating a similar microenvironment.

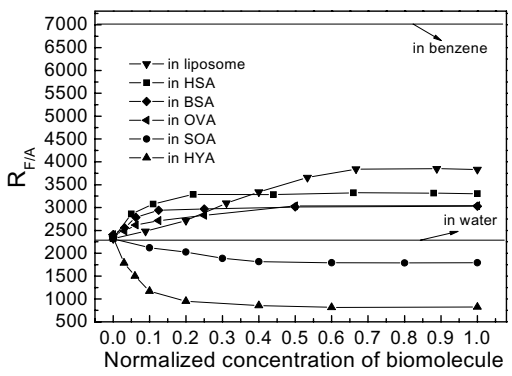


Fig. 9. $R_{F/A}$ values of HB (8 μM) in benzene solution, water and plot of $R_{F/A}$ values of HB (8 μM) as a function of normalized concentrations of liposome, HSA, BSA, OVA, SOA and HYA in PBS (pH = 7.0).

Compared to other kinds of photosensitizers whose fluorescence is quantitatively proportional to the absorbance, hypocrellins exhibit very distinct fluorescent feature, which may be quantitatively used to recognize various biological microenvironments. It is well known that tumor and normal cells have quantitative differences in expression of some biological molecules,[11,12,78] therefore, recognizing various biological molecules by the quantitative spectral parameters of HB may provide a clue for quantitatively distinguishing the microenvironments of tumor cells from normal ones.

3.3. *pH-sensitive fluorescence of hypocrellins*

Compared to the normal tissues, tumor tissues exhibit lower pH values.[79] Previously, fluorescent responses of hypocrellins to pH values were investigated. Figure 10 showed the fluorescent spectra

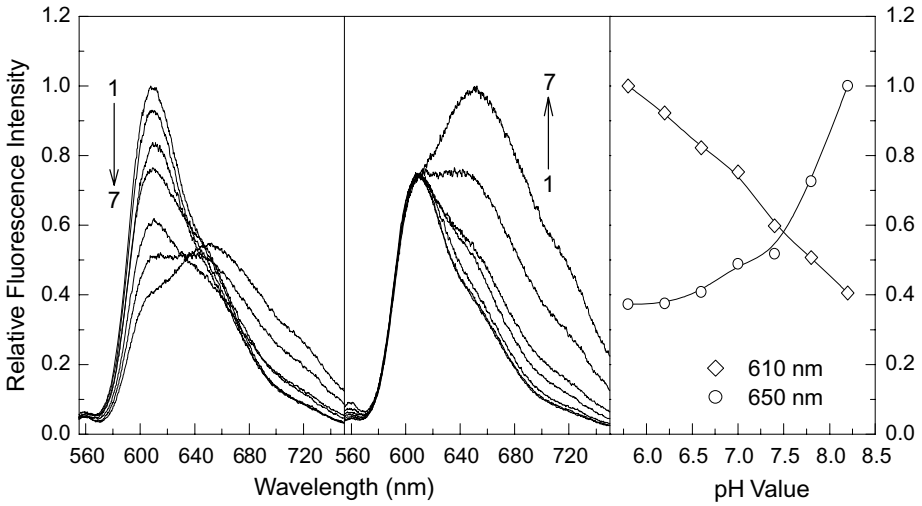


Fig. 10. (a) Fluorescence emission spectra of HB in HSA solution with various pH values from 5.8 to 8.2. (b) The normalized fluorescence spectra to the 610 nm peak. (c) pH-dependent fluorescent intensity at 610 and 650 nm.[73]

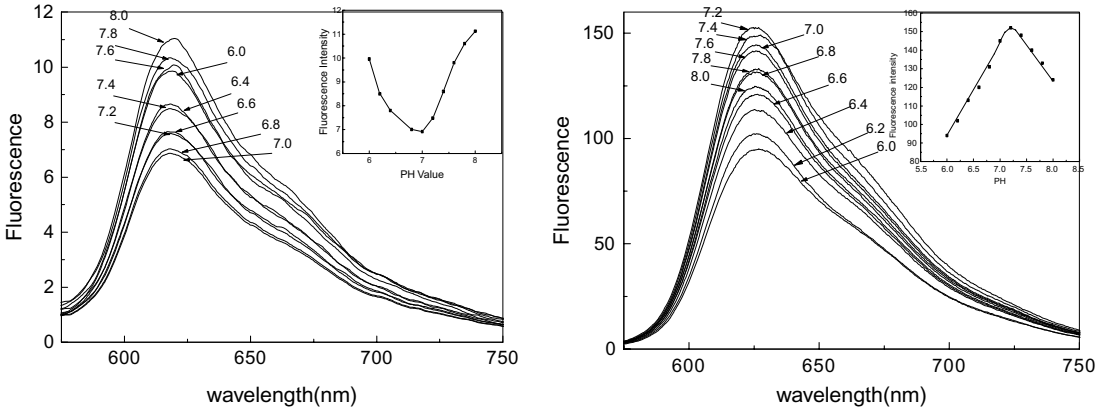


Fig. 11. Fluorescence spectra of HB in PBS (a) or in liposome (b) with a series of pH values. Insets: plots of peak fluorescence intensity to pH value.[80]

of HB binding to HSA in PBS with a series of pH values. In Figs. 10(b) and 10(c), the fluorescent spectra normalized to the 610 nm peak and changes of the fluorescence intensity at 610 and 650 nm quantitatively characterized the pH-dependent fluorescence of HB in HSA.

The fluorescence spectra of HB in PBS or in liposome were also monitored in a series of pH values from 6.0 to 8.0.[80] Interestingly, the fluorescence of HB in PBS exhibited the minimum at pH 7.0 while the maximum at pH 7.4 in liposome, as shown in Fig. 11, indicating that HB fluorescence is quantitatively sensitive to pH values in various microenvironments.

4. Conclusions and Perspectives

Generally, the drug-delivery problem for hypocrellins was solved by two strategies — preparing drug-delivery vehicles and finding quantitatively amphiphilic derivatives. Among the drug-delivery vehicles, liposomal powder formulation is most practical, for not only its PDT activity and stability, but also facile manufacture on a large scale. On the other hand, nanoparticles of HB with proteins or polysaccharides are stable and PDT effective, but controlled-release drugs make it not suitable for PDT of microvascular diseases. The nanoemulsion of hypocrellins is the most PDT effective, but its low stability does not satisfy the pharmaceutical standards. Commonly, all

of the drug-delivery vehicles exhibit lower PDT activity than the parents, HA or HB. As a new strategy, finding the derivatives with quantitative amphiphilicity and optimized photosensitization activity, not only solves the drug-delivery problem of hypocrellin photosensitizers, but also achieves higher PDT activity than the parents. In consideration of the spectral properties of hypocrellins and characters of the diseased targets, it can be concluded that the amphiphilic hypocrellin derivatives may be suitable for PDT of not only some microvascular diseases, but also other kinds of superficial diseases. Joint usage of PDD and PDT to treat tumors at an early stage may be a correct strategy to cure various tumors finally, however, to specifically differentiate a tumor cell or tissue from normal one by PDD is still a great challenge, because of lack of not only a specific marker from tumors, but also specific fluorescence of a photosensitizer. It is well known that over-expression of some biological molecules in tumor cells or tissues are a character of tumors, compared to normal cells or tissues. However, these are quantitatively but not qualitatively specific, that is, these molecules are also present in normal cells or tissues. Since presence of the quantitative differences between tumor and normal cells or tissues, logically, it may be asked whether the quantitative fluorescence responses can be used to recognize tumors. Particularly, hypocrellin fluorescence is environment-sensitive due to the intramolecular proton transfer mechanism, which is very distinct from other kinds of photosensitizers. As mentioned above, a specific spectral parameter of hypocrellins could not only recognize various kinds of biological molecules, but also identify a specific binding or nonspecific binding with same kind of biological molecules. These may provide a possibility to quantitatively differentiate the microenvironments of tumor from normal cells or tissues, however, further studies are certainly necessary to achieve the final goal.

Acknowledgments

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