The spectra character of photodegraded the pyridinoline cross-links by Hypocrellin B

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Abstract. Pyridinoline cross-links is one of the cross-link formation in collagen which in cell matrix, many research shown that this cross-link cause the fibrosis. Hypocrellin B (HB) is one of the nature photosensitizers, this work investigated the pyridinoline cross-link in collagen was photodegraded by HB. The result shown HB can degrade the pyridinoline cross-link with photo. This is to say, HB may be use as the photodynamic reagent to study the fibrosis.

1. Introduction

Hypocrellin B (HB), the structure as figure 1, was quantitatively produced by the dehydration of HA which was extracted from the fungus of Hypocrella bambusae and crystallized twice from chloroform-petroleum ether before use \(^1\). It is well known for the high photodynamic activity to tumors and other biological targets but very low dark-toxicity \(^2-4\).

The pyridinoline cross-link is a fluorescence group in collagen and gelatin, and the pyridinoline cross-link formation in collagen so it can’t degrade by metalloprotease, then collagen over-amount accumulated in tissues which cause fibrosis \(^5, 6\). Some research found the Photodynamic therapy (PDT) could prevent the vascular restenosis after surgery and effectively control the postoperative fibrosis in human glaucomatous eyes \(^7\). It was also reported that the pyridinoline cross-link in the fibrotic skin could be photo-degraded by psoralen under the ultraviolet light \(^8, 9\).

In this work, the spectrum character of the photoreaction of collagen with HB was investigated, the results shown the pyridinoline cross-link in collagen and gelatin could be photo-degraded by hypocrellin B, the reaction system was saturated by O\(_2\), Air and Ar respectively.

![Fig. 1 the structure of hypocrellin B (HB)](image-url)
2. Materials and methods

2.1. Materials
HB was got by Hypocellin A which was extracted from the fungus of *Hypocrella bambusae* and crystallized twice from chloroform–petroleum ether before use. Collagen (type I, c9879) was purchased from Sigma and was used as the received. The phosphate-buffered saline (PBS) of pH = 7.4 was prepared by using 50 mmol/L KH$_2$PO$_4$ and 50 mmol/L K$_2$HPO$_4$. The working solutions were prepared shortly before use.

2.2. The photoreaction
The HB-collagen solution was oxygen saturated or bubbled with nitrogen according to the experimental requirements, and then irradiated in a 1 cm×1 cm quartz cuvette. A 450 W mercury vapor lamp equipped with a filter cutting off the wavelengths shorter than 450 nm was used for irradiation. The fluence rate was about 10 mW/cm$^2$ measured by a UV-radiometer.

2.3. Spectroscopy measurements
The absorption spectra were recorded with a 4802s UV/Vis spectrophotometer (Shanghai Unico, China). The F-7000 fluorescence (Hitachi, Japan) was employ to investigate the fluorescence. Samples were dark-adapted at room temperature for 5 min prior to measurement.

3. Results

3.1. The spectrum of Collagen in solution
The collagen was been solved in PBS (pH = 7.4), the UV-Vis and Fluorescence Spectroscopy of the type I collagen in aqueous were detected in this paper. The results were shown in figure 2.

![Absorption and Fluorescence Spectra](image)

Fig. 2 The spectra character of the collagen A is UV spectrum, B is the Fluorescence spectrum.

The UV spectrum shown the type I collagen had the strong absorption at 227nm. The collagen solution was excited at 295nm, the fluorescence spectrometer detected the 395nm fluorescence emission. The 295/395nm fluorescence is the characteristic of pyridinoline cross-link in type I collagen. So the 395nm fluorescence peak could be as the fluorescent probe to detect the pyridinoline cross-link in collagen.

3.2. The photoreaction of HB with collagen
The photoreaction of hypocrellin B with collagen irradiated by 450W mercury vapor lamp was researched by UV-Vis Spectroscopy and F-7000 fluorescence spectrometer. The experiment found that, one hand, the pyridinoline cross-links can be degraded by the photosensitivity of HB, on the other hand, the pyridinoline cross-links was photodegraded faster when HB and O$_2$ all exist in the system than one of them absent. The ability of photodegraded of HB was shown in figure 3.

![Absorption spectra](image1)

![Absorption spectra](image2)

![Absorption spectra](image3)

![Absorption spectra](image4)

Fig.3 The Absorption spectra of the photo-reaction of collagen with irradiation of HB for 0,1,2,5,10,15,20 and 25min in solution saturated by argon (a), air(b) and oxygen(c) respectively. (d) Plot of the 395nm fluorescence intensity to irradiation time.
Fig. 4 The fluorescence spectra of the photo-reaction of collagen with irradiation of HB for 0, 1, 2, 5, 10, 15, 20, and 25 min in solution saturated by air (a), argon (b), and oxygen (c) respectively. (d) Plot of the 395 nm fluorescence intensity to irradiation time.

Figure 3 shows the Absorption of HB in collagen solution with the irradiation. This figure suggested the photoreaction mechanism is main via the type I mechanism. The existence of collagen can ‘protect’ the HB from being photobleached.

Figure 4 shows changes of the fluorescence of pyridinoline cross-link in collagen with HB during the irradiation. The result shown the pyridinoline cross-link was photodegraded when the HB existed in the system with irradiation. It can be seen that the 395 nm fluorescence quenching, reflecting degradation of pyridinoline cross-link, was much more effective in the presence of oxygen. So the photo-degradation speed of pyridinoline cross-link dependent on oxygen.

The ROS are the most important reactive species for the photoreaction (Type II mechanism) when the oxygen presence. The type I mechanism is the main way to degrade this cross-link when the oxygen absent from the reaction system.

4. Conclusions
In this paper, the 295/395 nm fluorescence was ascribed to the pyridinoline cross-link formation in collagen. The result shown the level of pyridinoline cross-link in type I collagen could be reduce by HB photodynamic action under irradiation by mercury vapor lamp. The existence of collagen can ‘protect’ the HB from being photobleached. This suggesting the HB may use as photodynamic reagent for research the fibrosis in future.

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