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Research Article

Preparation and Characterization of Phycobiliprotein Mcirocapsule

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Abstract: Phycobiliprotein is a kind of bioactive material that is widely used in the fields of food and medicine. However, the usage of phycobiliprotein is limited by its instability. In this study, we firstly developed a kind of phycobiliprotein microcapsulate by chitosan-sodiumalginate encapsulation method. The preparation conditions, stability and controlled release of phycobiliprotein microcapsule were studied and discussed. The results showed that the optimal conditions for preparing the microcapsulates were 3% sodium alginate, 3% $CaCl_2$ and 0.5% chitosan. After encapsulation, the stabilities of phycobiliprotein to heat, acidity and radiation were greatly improved. Phycobiliprotein in microcapsulate was 5-10 times more endurable than in water solution when heated and 7 times when radiated. Stability under acidity was also improved greatly. It was also found that phycobiliprotein microcapsulate had good stability in the mimic gastric juice and had 90% release rate in the mimic intestinal juice. This suggests that phycobiliprotein microcapsulate can have controlled release and dissolution in intestine.

Keywords: Controlled release, microcapsule, phycobiliprotein, stability

INTRODUCTION

Phycobiliprotein is a common light-harvesting protein of algae. As a natural pigment protein, phycobiliprotein has good potential application in food coloring and cosmetics (José *et al*., 1995). It can also eliminate free radicals and active oxygen, kill tumor cells and increase lymphocyte activity. Therefore, phycobiliprotein has broad application in the pharmaceutical industry (Sekar, 2008). However, instability of phycobiliproteins under light or heat would greatly limit its application.

Microencapsulation is using natural or synthetic polymer materials to enclose core materials in a tiny capsule. It can protect the encapsulated material from the external environment, thus greatly improving the stability of encapsulated substances (Gombots and Wee, 1998; Liu *et al*., 2007). Therefore, phycobiliproteins in the form of microcapsules would enhance its stability, extend its biological activity and improve its preservation and use. However, the microencapsulation of phycobiliproteins has not been reported so far.

In this study, phycobiliproteins and chitosan were extracted. Phycobiliprotein microcapsules were made by the encapsulation method of chitosan-sodiumalginate. Stability and controlled release of microcapsulated phycobiliprotein in simulated gastric and intestinal fluid were studied and discussed, which can be used for further research and development of phycobiliproteins.

MATERIALS AND METHODS

Materials and drugs: Spirulina powder (Xiamen Junyuan Biotechnology Co., Ltd.); chitosan crude (Xiamen Huading Animal Health Products Co., Ltd.); sodium alginate (chemically pure); other drugs were of analytical grade.

Preparation of phycobiliprotein microcapsules: First, phycobiliprotein was extracted from spirulina powder (Zhang *et al*., 1999) and chitosan powder was extracted from the crude chitosan (Zhang *et al*., 1999; Zeng *et al*., 2002). Then chitosan and $CaCl_2$ were dissolved with 5% acetic acid to make the acetic acid solution of chitosan-$CaCl_2$ (chitosan: 0.5%; $CaCl_2$: 3%). Phycobiliprotein-sodium alginate aqueous solution was prepared (sodium alginate: 3%; phycobiliproteins: sodium alginate = 1:6) and dropped into the chitosan-$CaCl_2$ solution using a needle. After reaction and washing, phycobiliprotein microcapsules can be collected by centrifugation and vacuum drying.

Determination of phycobiliprotein concentration: According to the method described in literature (Siegelman and Kycia, 1978), the concentration of phycobiliprotein in aqueous solution can be determined.

Optimization of microcapsule preparation: Three concentration gradients of sodium alginate (2, 3, 4%), $CaCl_2$ (1, 3, 5%) and chitosan (0.1, 0.3, 0.5%),

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respectively were studied to determine optimal conditions based on microcapsule shape and embedding rate.

Determination of microcapsule embedding rate: A certain amount of dried microcapsules was weighted and placed into 0.1 mol/L sodium citrate solution until the complete rupture of microcapsules. The insoluble matter was filtered. Based on the concentration of phycobiliprotein in the solution determined as describe previously, the Content of Phycobiliprotein (DCP) in microcapsule can be calculated (Zhang and Hao, 1997). In addition, the Theoretical Content of Phycobiliprotein (TCP) in microcapsule was determined as followed:

$$TCP \text{ in } microcapsules = \frac{Total\ amount\ of\ phycobiliprotein}{Total\ amount\ of\ microcapsule} \times 100\%$$

Thus, the Embedding Rate (ER) was calculated as ER = DCP/TCP.

Stability of phycobiliprotein microcapsules:

- **Stability of phycobiliproteins to heat:** Both phycobiliprotein microcapsules and phycobiliprotein solutions (pH = 6.8) were prepared with equivalent amount of phycobiliproteins. Samples were then heated on 25, 40, 60, 80, 100°C, respectively under dark condition. At regular intervals, phycobiliprotein microcapsules were broken by sodium citrate and scanned using DU640 spectrophotometer in the 200-800 nm. Phycobiliprotein solutions were directly scanned to attain UV-visible absorption spectra. Deterioration of phycobiliproteins was calculated and compared.
- **Stability of phycobiliproteins to acidity:** Aqueous solutions of pH values 0-14 were prepared. Phycobiliprotein microcapsules and phycobiliprotein powder were dissolved respectively in solutions with the same pH value at room temperature (25°C) under dark condition. Deterioration of the phycobiliprotein was measured and compared at regular intervals. Experiment lasted 7 days.
- **Stability of phycobiliproteins to radiation:** Similar solutions were prepared at pH = 6.8 and room temperature (25°C) under natural light exposure. Stabilities were similarly measured and compared under natural light condition. Experiment lasted 7 days.

In vitro release test:

- **Controlled release in the mimic gastric juice:** Mimic gastric juice 20 mL was prepared and maintained at 37°C (Zhang and Hao, 1997; Fordtran and Lockear, 1996). Phycobiliprotein microcapsules with known content were dissolved in the mimic gastric juice. Phycobiliprotein will degenerate in the acid environment of gastric juice and cannot be directly measured. But we can measure residual capsules after centrifuge at different times and calculate the amount of residual phycobiliproteins to get the Release Rate (RR):

$$RR\ (\%) = \frac{\text{Initial amount of phycobiliproteins of microcaosules} - residual\ amount\ of\ phycobiliproteins}{\text{Initial amount of phycobiliproteins of microcaosules}} \times 100\%$$

- Controlled release in the mimic intestinal juice: Mimic intestinal fluid 20 mL was prepared (Zhang and Hao, 1997; Fordtran and Lockear, 1996). Measurement was similarly conducted to calculate the Release Rate (RR):

$$RR\ (\%) = \frac{Amount\ of\ phycobiliproteins\ released\ in \text{ int}estrial\ fluid}{\text{Initial amount of phycobiliproteins of microcapsules}} \times 100\% + the\ RR\ in\ mimic\ gastric\ fluid$$

RESULTS AND DISCUSSION

Preparation conditions of phycobiliprotein microcapsule: In alginate-chitosan method, shape and embedding rate of microcapsules are closely related to concentrations of sodium alginate, chitosan and $CaCl_2$. It was found that 2% sodium alginate would make microcapsules have serious tailing phenomenon, or even fail to form. Also 4% sodium alginate would always plug the needle. Therefore, 3% sodium alginate was chosen for the preparation of the microcapsule.

$CaCl_2$ also has influence on the shaping of microcapsules. Study showed that 3% $CaCl_2$ would make the microcapsule with uniform and spherical shape, but 1 and 5% $CaCl_2$ would make the microcapsule with various sizes and non-spherical shapes. Therefore, 3% $CaCl_2$ was chosen for the microcapsule preparation.

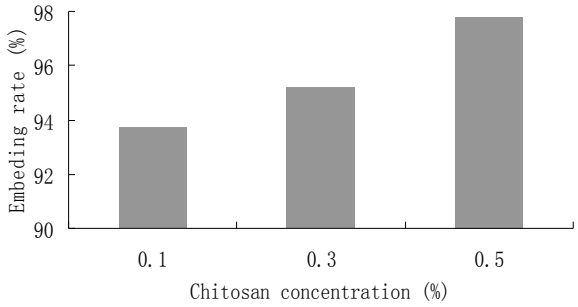

Fig. 1: Effect of the chitosan concentration on the embeding rate

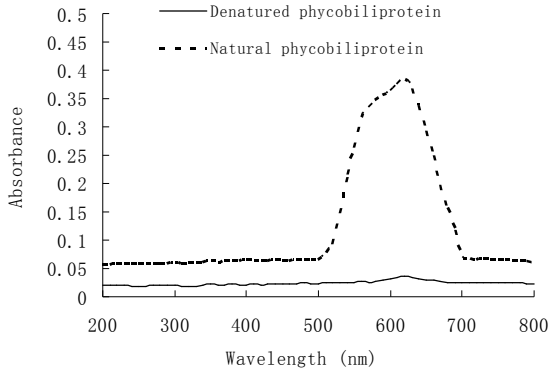

Fig. 2: Absorbance spectrum of the natural and denatured phycobiliprotein

Chitosan concentrations and embedding rates are correlated positively, which can be seen in Fig. 1. However, 0.5% chitosan would reach the maximum solubility in the solution for preparing microcapsules whose embedding rate was 97.8%. A further increase of chitosan concentration would be unnecessary for the extremely high encapsulation rate.

Currently, major methods of using sodium alginate and $CaCl_2$ to prepare microcapsules are dripping method, impregnation method and coacervation method. Dripping is mostly used. The key to the method is the control of sodium alginate and $CaCl_2$ concentration. Ma *et al*. (2003) showed that, when alginate is less than 1% or $CaCl_2$ is less than 0.02 mol/L, the drop cannot be made to the ball, which is consistent with our results. The choices of chitasan concentration are different among different reports (Sun *et al*., 2005), which may be related to the quality of raw materials and chitosan crystal.

Stability of phycobiliprotein microcapsule: Undenatured phycobiliprotein has absorption peak at 620 nm while denatured don't have any peak (Fig. 2), which can be used to determine whether phycobiliproteins is denatured or not.

Microencapsulated phycobiliproteins have stronger tolerance to heat, pH and light than phycobiliproteins in solution (Fig. 3). Figure 3a shows that microcapsules of phycobiliproteins can be maintained at 40°C for 140 min while phycobiliproteins in solution can only be maintained for 15 min. Even under the high temperature of 100°C, phycobiliproteins in solution can be maintained for 1 min while micro-capsulated phycobiliproteins can be maintained for 5 min. At room temperature 25°C, the solution can be maintained for only 1 day while phycobiliprotein microcapsules can be maintained for 5 days (not shown). It shows that thermal stability of phycobiliprotein microcapsules has been greatly improved.

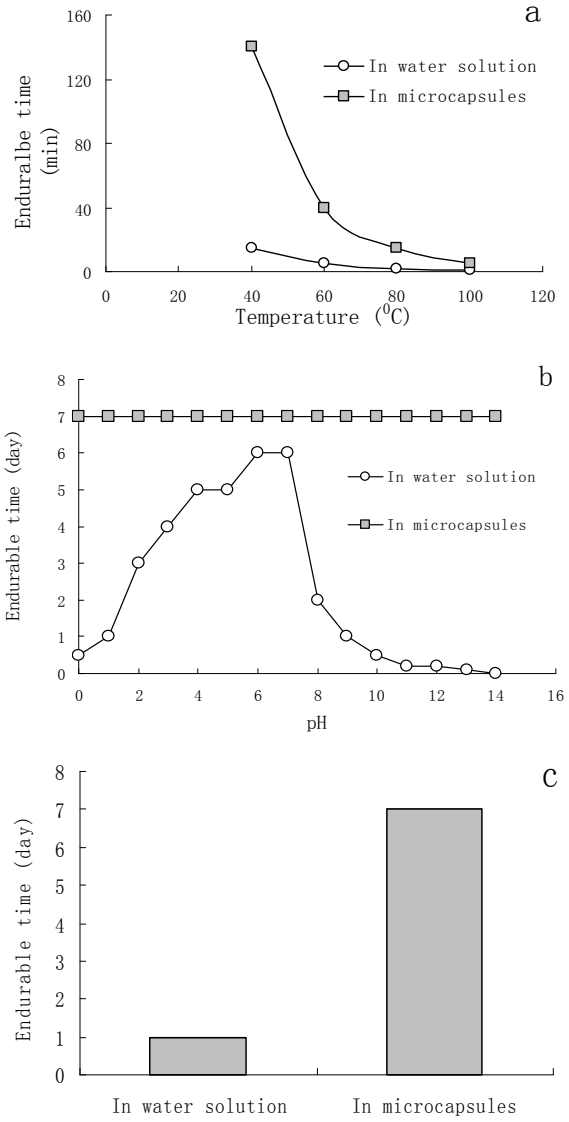

Fig. 3: Comparison of stabilities of phycobiliprotein in water solution and in microcapsules to heat (a), pH (b) and illumination (c)

Phycobiliprotein in solution is unstable in alkaline and strong acidic environment; and degeneration appeared within 3 days. Even in the neutral

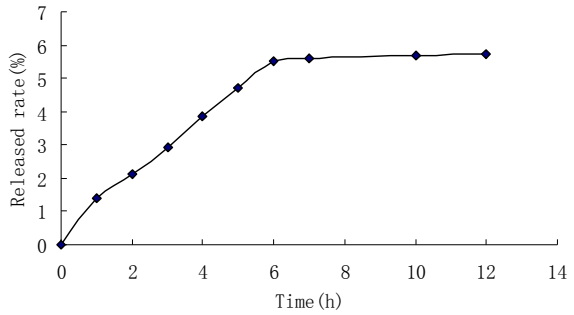

Fig. 4: Release rates of phycobiliprotein microcapsules in the mimetic gastric juice

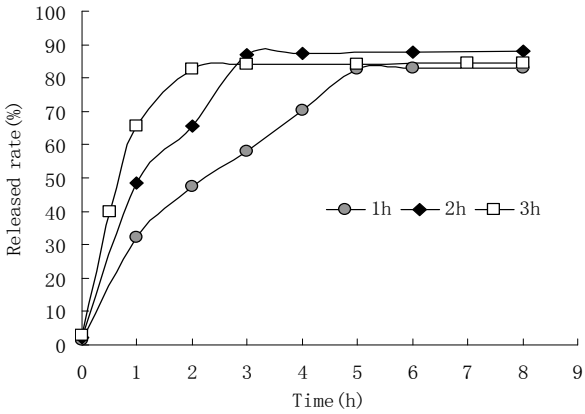

Fig. 5: Release rates of phycobiliprotein microcapsules in the mimic intestinal juice after treated in the mimic gastric juice for 1, 2 and 3 h, respectively

environment (pH = 6-7) that is more stable, degeneration appeared in the first 6 days (Fig. 3b). On the other hand, phycobiliprotein microcapsules can be maintained in a variety of pH for 7 days without degeneration (Fig. 4), which indicates that phycobiliprotein microcapsules have significantly improved tolerance for pH disturbance.

Phycobiliproteins in solution degenerate within one day under room temperature and natural light condition. Phycobiliprotein microcapsules under the same conditions remained stable after 7 days without significant degeneration (Fig. 3c).

Stability of microencapsulated active substance is usually greatly improved. It is mainly due to the protective effect of capsule wall (Czeczuga, 1985). Microcapsule can protect the material from the external environment, thereby delaying or preventing external environmental factors from interacting with encapsulated materials.

In vitro release tests: The release rate in mimic gastric juice of microcapsules gradually increased over time, reaching stability after 6 h (Fig. 5). The final release rate is less than 6%, which indicates that phycobiliprotein microcapsules have higher stability in acidic gastric juice. In the mimic intestinal fluid, the release rate also gradually increased over time. The longer the microcapsules were treated in mimic gastric juice, the faster phycobiliprotein released in mimic intestinal fluid. Phycobiliprotein microcapsules were treated for 1, 2, 3 h, respectively in the mimic gastric fluid with release rate reaching stability after 6, 4, 2 h, respectively in simulated intestinal fluid, which indicates that strong acidity of gastric juice has damaging effects to the surface membrane of microcapsules. The final release rate of microcapsules in mimic intestinal fluid was close to 90%, which indicated that most phycobiliproteins were released in the intestinal fluid. This is good for our body to effectively absorb phycobiliprotein.

CONCLUSION

This study for the first time developed phycobiliprotein microcapsules that have slow-release control. Optimal conditions for the preparation were determined: 3% sodium alginate, 3% $CaCl_2$ and 0.5% chitosan. Stabilities of phycobiliprotein microcapsule to heat, light and pH were substantially improved. The heat tolerance was 5-10 times as in solution; light tolerance was 7 times more; and pH tolerance was greatly improved.

In mimic gastric juice, phycobiliprotein microcapsules had stabilized release rate below 6% while they had final release rate close to 90% in mimic intestinal juice. This shows that phycobiliprotein microcapsule has slow-release in intestines. In addition, the release rate of phycobiliprotein microcapsules in bowel liquid is positively correlated to of the length of its stay in gastric juice, suggesting that stomach's acid environment can destroy the microcapsule.

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