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The preparation and stability of the inclusion complex of astaxanthin with β-cyclodextrin

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Abstract

Inclusion complex of astaxanthin with β-cyclodextrin was prepared. The water solubility of the inclusion complex was <0.5 mg/ml, which is better than that of astaxanthin. Large aggregates were observed in the aqueous solution of the inclusion complex. Furthermore, the stability of the inclusion complex against temperature and light was greatly enhanced compared to that of astaxanthin.
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Keywords: Astaxanthin; β-Cyclodextrin; Inclusion complex; Stability

1. Introduction

Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione) is a carotenoid pigment found in certain marine animals and plants such as fish, shrimp and algae (Boussiba, Fan, & Vonshak, 1992). It can be obtained from synthetic and natural sources, e.g. microalgae, yeast, shrimp, crab or lobster by-products. The Hoffman-La roche Inc. has synthesized all-*trans* astaxanthin (Fang & Chiou, 1996). However, the biological function of synthesized astaxanthin is less than that of natural astaxanthin (Wei & Yan, 2001).

The most important commercial application of astaxanthin is in the aquaculture industry where it is used in the formulation of feed for farmed salmon to provide the typical muscle colour, which is widely accepted by consumers throughout the world. Astaxanthin has several key biological functions in fish. It is associated with reproduction and embryo development and also with protecting cells against oxidative damage (Parajo, Santos, & Vazquez, 1996; Putnam, 1991). In human nutrition, astaxanthin has been gaining widespread popularity as a dietary supplement due to its powerful antioxidant properties. Currently, several astaxanthin products derived from microalgae are available in the marketplace, and being promoted as anticancer and antiinflammatory agents as well as immunostimulants (Ciapara, Valenzucla, Goycoolea, & Monal, 2004). The powerful antioxidant property of astaxanthin is due to its special structure, with a total of 11 conjugated carbon–carbon double bonds. The antioxidant property of astaxanthin is 10 times stronger than that of β-carotene, and up to 500 times stronger than vitamin E (Shimidzu, Goto, & Miki, 1996). Because astaxanthin is a highly unsaturated molecule, it can easily be damaged by heat or light, which can cause the loss of biological properties. Furthermore, the poor aqueous solubility of astaxanthin limits its use as an aqueous-phase antioxidant.

Recently, β-cyclodextrin(β-CD) is widely used to prepare inclusion products in food and pharmaceutical science. The inclusion of β-CD with the guest molecules can change physical and chemical properties of the guest molecules, such as increasing aqueous solubility and stability. Much interest has been concentrated on preparing inclusion complexes of β-CD with various small molecules

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(Mele, Mendichi, & Selva, 2002). Many substances e.g. drugs (Hedges, 1998; Liu & Fan, 2005; Szejtli, 1988; Takahaski, 1998; Tenjarla, Puranajoti, Kasina, & Mandal, 1998), and pigments (Sun, Li, & Ding, 2002), have been made into inclusions of β-CD. There are very few studies using β-CD to prepare the inclusion of astaxanthin. In this paper, we report the preparation of inclusion complex between β-CD and astaxanthin, and measurement of aqueous solubility and stability of the resultant complex against heat and light.

2. Materials and methods

2.1. Chemicals

According to Chen, Huang, and Ouyang (2005), astaxanthin was prepared from dried small shrimps in our laboratory. β-CD (98%) was purchased from Beijing Ao Bo Xing Biochemical Corporation (Beijing, China). All other reagents were of analytical grade. The water used was double-distilled and deionized.

2.2. The preparation of the inclusion complex of astaxanthin with β-cyclodextrin

Astaxanthin (0.5 g) was dispersed into dichloromethane/acetone (1:1, v/v, 20 ml). β-CD (2 g) was dissolved in distilled water (20 ml) with stirring at 50 °C. Then, the solution of β-CD was added to the solution of astaxanthin. The mixture was stirred for 7 h at 50 °C. After cooling to room temperature, the mixture was stored at 4 °C for 24 h. Afterwards, it was filtered under vacuum. The deposit was washed 3 times with ethanol and distilled water, respectively. Finally, the deposit was dried under vacuum for 24 h. The IR spectrum of the inclusion complex is shown in Fig. 1.

2.3. Quantitative analysis of astaxanthin in the inclusion complex

Astaxanthin (98%, 10 mg) was dissolved into methanol (100 ml). The solution (10 ml) was then diluted respectively, with the final concentrations of 2, 4, 6, 8, 10, 12 and 14 μg/ml. These samples were analyzed by HPLC according to Jordi and Palou (2000). The HPLC system used consisted of a Waters 515 pump (Waters, Milford, MA, USA), a sample injector (Rheodyne, Cotati, CA, USA) with 20 μl loop, and a Waters 996 photodiode array detector. Evaluation and quantification were made on a Millenium chromatography data system (Waters). The column used was a C_{18} column (300 × 3.9 mm, ID, 5 μm, Beckman, Fullerton, CA, USA). The mobile phrase was methanol–water (9:1, v/v). The flow rate was 1.5 ml/min and the effluent was monitored at 475 nm. The area under the peaks of astaxanthin was calculated. Then, the regression equation was made between the areas (Y) and the concentrations (X) of astaxanthin.

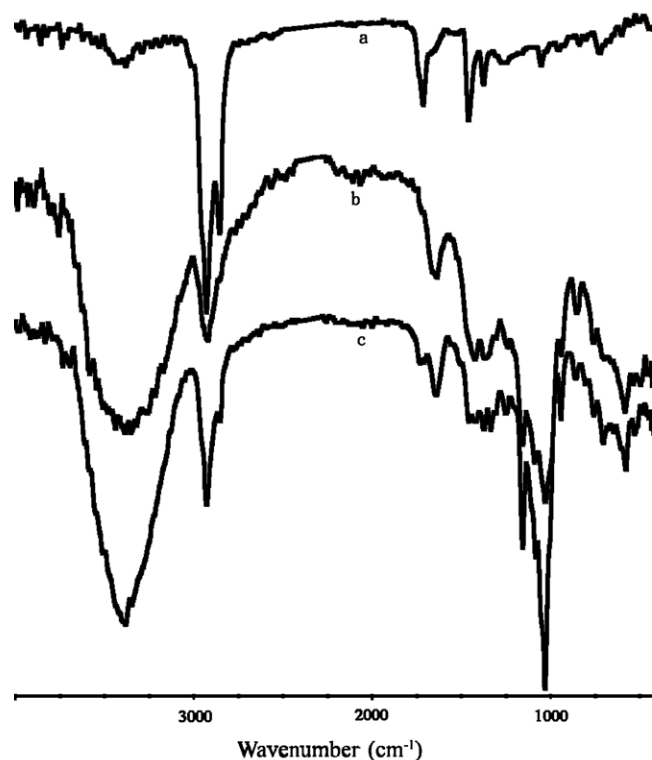

Fig. 1. The IR spectra of astaxanthin (a), β-CD (b) and the inclusion complex (c).

The inclusion complex of astaxanthin with β-CD (10 mg) was dissolved into methanol (100 ml). The solution was put under ultrasonic environment for 10 min. This process was repeated six times. Then, the solution was analyzed by HPLC. The area under the peak of astaxathin was calculated.

2.4. The aqueous solubility of the inclusion complex

The encapsulation (10 mg) was dispersed into distilled water (20 ml). The solution was turbid. After 6 h, floc appeared in the solution. When ethanol (20 ml) was added to the solution, the floc disappeared.

2.5. The stability of the inclusion complex

The inclusion complex was divided into four groups. Three of them were stored at 4 °C, 30 °C, 57 °C and under light (with light intensity 1500 Lux), respectively. The inclusion complex (30 mg) and astaxanthin were dissolved in acetone/n-hexane (1:1, v/v, 20 ml), respectively. The absorbance of the mixture was measured at 480 nm. This experiment was repeated after 6 days.

2.6. Statistical analysis

All determations were carried out in triplicate. All data are expressed as mean ±SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated

by Duncan's multiple range tests. The results were processed using Excel and statistical software (1999).

3. Results and discussion

In the IR spectrum (as shown in Fig. 1) of the inclusion complex(c), characteristic absorbance at 1736 cm^{-1} was assigned to C=O bond in the molecule of astaxanthin. The peak at 940 cm^{-1} in the spectrum of c was greatly enhanced, compared with that of β-CD(b), which showed that the framework vibration in the molecule of β-CD was disturbed. The peak at 1028 cm^{-1} was greatly enhanced, which showed that bend vibration of O—H in the molecule of β-CD was increased. The peak at 1156 cm^{-1} was also greatly enhanced, which showed that the C—C bond was stretched in the ring of β-CD. These results might be caused by the effect of the long π-bond in the astaxanthin molecule and the formation of H-bond between the molecule of astaxanthin and the molecule of β-CD. This indicated that the inclusion complex of astaxanthin with β-CD was obtained.

The results of HPLC showed that, when the concentrations of astaxanthin were from 2 μg/ml to 4 μg/ml, there was good linear relationship between the concentrations of astaxanthin and the areas below the peaks of astaxanthin. The regression equation was as follows:

$$Y = 275,473X - 1013.1, \quad R = 0.9994.$$

X – The concentrations of astaxanthin

Y – Area under the peak of astaxanthin

The result of HPLC for the disposed inclusion complex showed that the area below the peak of astaxanthin was 3304662. Therefore, according to the regression equation, the concentration of astaxanthin in the disposed inclusion complex was 12 μg/ml. From the result of 12 μg/ml, the equation was founded as follows:

$$\frac{596.86}{1135 \times N + 596.86} = \frac{12 \times 10^{-3} \times 100}{10}$$

596.86 – Molecular weight of astaxanthin

1135 – Molecular weight of β-CD

N – Number of β-CD including one molecule of astaxanthin.

The result of the equation afforded $N = 4$. Thus, the complex is 1:4 for astaxanthin:β-cyclodextrin. In all, 2.04 g of the inclusion complex were obtained. According to the following equation,

$$\text{Rate of inclusion (\%)} = \frac{12 \times 10^{-6} \times 100 \times 2.04/(10 \times 10^{-3})}{0.5} \times 100$$

The formula of $12 \times 10^{-6} \times 100 \times 2.04/(10 \times 10^{-3})$ is the quantity of astaxanthin in the inclusion complex (2.04 g). Finally, the rate of inclusion (%) was 48.96%.

The aqueous solubility of the inclusion complex (<0.5 mg/ml) was slightly better than that of astaxanthin. The fact that floc appeared in the solution of the inclusion complex after 6 h, showed that the inclusion complex likely formed self-assemblies in an aqueous environment. When ethanol was added to the solution, the floc disappeared which showed that monomer of the inclusion complex was formed. This result agrees with that of Bikadi, Zsila, Deli, Mady, and Simonyi (2002).

As shown in Figs. 2–5, within 6 days, the absorbance of astaxanthin (a) reduced rapidly at 4 °C, 30 °C and 57 °C. Furthermore, the absorbance of astaxanthin at higher temperatures was reduced more rapidly than that at lower temperatures. However, the absorbance of the inclusion complex (b) increased slowly within 6 days. Although the absorbance of the inclusion complex at 57 °C increased, it was not statistically significant ($P < 0.05$). These results show that the effect of temperature on the inclusion complex was minimal, which was different from that observed for astaxanthin. Hence, when astaxanthin and β-CD were formed into inclusion complex, the heat stability of astaxanthin was greatly enhanced.

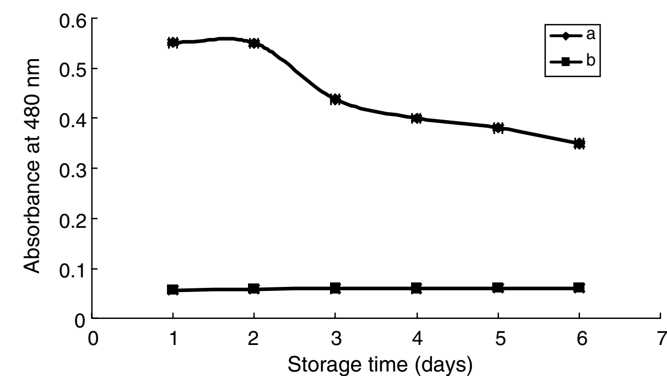

Fig. 2. The absorbance of astaxanthin and the inclusion complex in acetone/*n*-hexane (1:1, v/v) within 6 days at 4 °C.

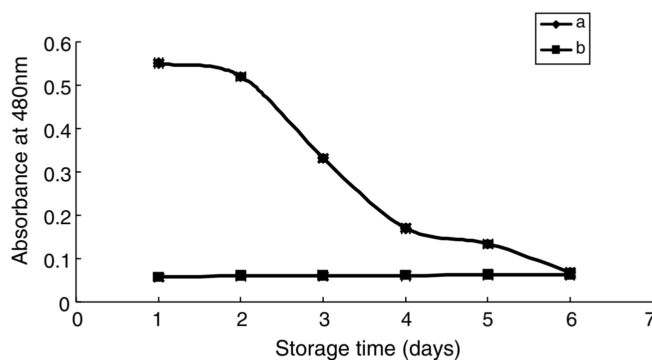

Fig. 3. The absorbance of astaxanthin and the inclusion complex in acetone/*n*-hexane (1:1, v/v) within 6 days at 30 °C.

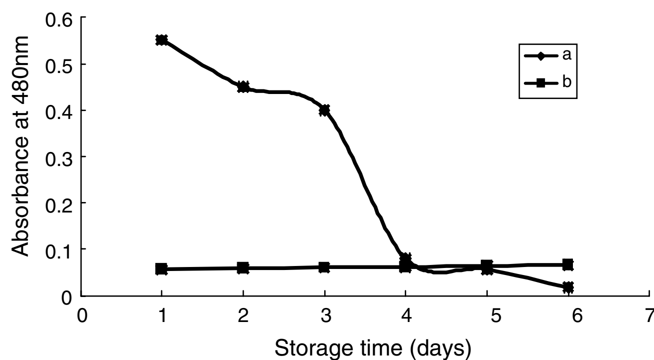

Fig. 4. The absorbance of astaxanthin and the inclusion complex in acetone/*n*-hexane (1:1, v/v) within 6 days at 57 °C.

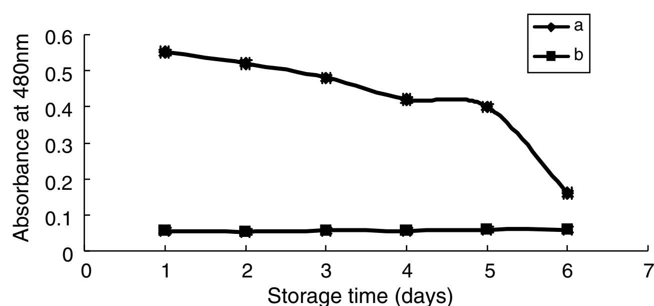

Fig. 5. The effect of light on the absorbance of astaxanthin and the inclusion complex in acetone/*n*-hexane (1:1, v/v).

As shown in Fig. 5, within 5 days, the absorbance of astaxanthin was reduced slowly. On the sixth day, it reduced rapidly, whereas there was no obvious change of the absorbance of the inclusion complex. These results showed that the effect of light on the inclusion complex was insignificant, but the effect of light on astaxanthin was substantial. Hence, when the astaxanhin and β-CD formed inclusion complex, the stability of the inclusion complex against light was also greatly enhanced.

Astaxanthin is the main carotenoid pigment found in aquatic animals and is present in many of our favorite seafoods including salmon, trout, red seabream, shrimp, lobster and fish eggs. It is closely related to other well-known carotenoids, such as β-carotene, zeaxanthin and lutein, thus they share many of the metabolic and physiological functions attributed to carotenoids. Astaxanthin has several essential biological functions including protection against oxidation of essential polyunsaturated fatty acids, protection against UV light effects, immune response, pigmentation, communication, reproductive behaviour and improved reproduction. Therefore, it has important applications in the nutraceutical, cosmetics, food and feed industries. The supplementation of astaxanthin in food might be a practical and beneficial strategy in health management (Guerin, Huntley, & Olaizola, 2003; Lorenz & Cysewski, 2002). However, free astaxanthin is particularly sensitive to oxidation, so it is important to stabilize the free astaxanthin. The complex was 1:4 for astaxathin:β-cyclodextrin. The heat stability of the inclusion complex against and its stability under light were greatly enhanced. Furthermore, the aqueous solubility of the inclusion complex was slightly enhanced (<0.5 mg/ml). This conclusion might broaden the potential uses of astaxanthin.

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