

## THE INFLUENCE OF PROPOLIS INGESTION ON REDOX STATE OF HUMAN SERUM ALBUMIN: A STUDY OF PATIENTS UNDER SEVERE OXIDATIVE STRESS

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### Abstract

Human serum albumin (HSA) is a mixture of mercaptalbumin (HMA, reduced form) and nonmercaptalbumin (HNA, oxidized form). HMA, possessing a single sulfhydryl residue, is responsible for the largest fraction of reactive sulfhydryl in extracellular fluids and therefore it seems to participate largely in the extracellular antioxidant system. We have developed a convenient high-performance liquid chromatographic (HPLC) system for the clear separation from HSA to HMA and HNA. By this method, a mean value of [HMA/(HMA+HNA)], i.e. f(HMA) for healthy young male subjects was 73.2% (n = 20). In the present study, we examined the influence of propolis ingestion on the redox state of HSA from patients under severe oxidative stress such as a cancer therapy. In the case of a patient with liver cirrhosis and esophageal cancer (male, 68 yr), f(HMA) value before the radiation therapy without propolis ingestion was 66.6%. However, during the radiation therapy for 2 weeks, he took ten propolis tablets/day (525 mg of Brazilian propolis extract/day) every day and continued to take them for more 4 weeks. Values for f(HMA) just before, immediately after, and 4 weeks after radiation therapy, were 63.5, 67.7 and 74.1%, respectively. These evidences suggest that oxidative status due to the radiation therapy might be attenuated by propolis supplementation. Furthermore, a gradual increase in f(HMA) value indicates that the antioxidant properties of propolis might augment the total antioxidant capacity of the patient with cancer.

**Keywords:** Propolis / oxidative stress / redox state / serum albumin (human)

### Introduction

Human serum albumin (HSA) is the most abundant protein in the circulatory system and has a number of functions. It is well known that it plays an important role in the osmotic regulation of the circulating fluid within the vascular system and is able to transport a wide variety of endogenous and exogenous substances through the body (PETERS, 1996). Another functional role of this molecule seems to be a maintenance of the redox potential in the extracellular fluids, because it is a mixture of mercaptalbumin (reduced form; in humans, HMA) and non-mercaptalbumin (oxidized form; in humans, HNA), i.e., redox couple in plasma (SOGAMI et al., 1984; SOGAMI et al., 1985a; SOGAMI et al., 1985b). HMA has one free sulfhydryl group in position 34 (Cys-34) and is responsible for the largest fraction of free sulfhydryl in the extracellular fluids. HNA is composed of at least three kinds of compounds. The major HNA compound is mixed disulfide with cysteine or glutathione (HNA(Cys) or HNA(Glut)), and the other is an oxidation product higher than mixed disulfide, such as sulfenic (-SOH), sulfinic (-SO<sub>2</sub>H) or sulfonic (-SO<sub>3</sub>H) state (HNA(Oxi)) as very minor component in extracellular fluids (ERA et al., 1989).

We have developed a convenient high-performance liquid chromatographic (HPLC) system for the clear separation of HSA to HMA and HNA, using a Shodex-Asahipak GS-520H or an ES-502N column, and we have extensively studied dynamic change in the redox state of HSA in various pathophysiological states (SUZUKI et al., 1992; HAYAKAWA et al., 1997; HAYASHI et al., 2000; KAWAI et al., 2001; SOEJIMA et al., 2002; TOMIDA et al., 2003). From the results obtained, the fraction of HMA (f(HMA)) has been shown to be markedly decreased in various diseases compared with that in healthy subjects, whereas there has been almost no change in f(HMA) value of the healthy subjects.

The purpose of the present study is to investigate the influence of propolis ingestion on the redox state of HSA from patients under severe oxidative stress such as a cancer therapy, because propolis, one of the bee products, is considered to play a beneficial role as a supplemental antioxidant (BANSKOTA et al., 2001).

### Materials and Methods

Two patients with gastrointestinal cancer were studied. One patient (female, 56 years old) suffered from gastric cancer and had undergone curative gastrectomy. Another patient (male, 68 years old) suffered from both liver cirrhosis and esophageal cancer independently, and had received radiation therapy against esophageal cancer. A healthy subject (male, 45 years old) with no history of renal or hepatic diseases participated as a control in this study (one of the authors; H.I.). As a propolis supplement, Brazilian propolis extracted by ethanol was used (each propolis tablet contained 52.5 mg of propolis extract, Yamada Apiculture Center Inc., Okayama, Japan). Informed consent was obtained from each subject, and all procedures were performed in accordance with the Helsinki Declaration. Blood samples were obtained in

vacuum blood-collecting tubes (EDTA-2K, Terumo Co., Tokyo, Japan) and these were centrifuged for 20 min at 3,000 rpm in a Kubota 2100 centrifuge (Kubota Manufacturing Co., Tokyo, Japan). The resulting plasma was then subjected to pressure filtration by the use of Cosmonice filter (0.45  $\mu$ m, Nacalai Tesque., Inc., Kyoto, Japan), and specimens were stored at  $-80^{\circ}\text{C}$  until HPLC analysis.

The HPLC system consisted of a Model AS-8010 autosampler, a Model CCPM double-plunger pump and a Model FS-8000 fluorescence detector (excitation wavelength, 280 nm; emission wavelength, 340 nm) in conjunction with a Model SC-8020 system supercontroller, all from Tosoh Co., Japan. A Shodex-Asahipak ES-502N column (10 x 0.76 cm I.D., DEAE-form for ion-exchange HPLC, Showa Denko., Japan; column temperature,  $35.0 \pm 0.5^{\circ}\text{C}$ ) was used. Elution was carried out with a linear gradient of increasing ethanol concentration from 0 to 5%, in 0.05 M sodium acetate-0.40 M sodium sulfate (pH 4.85) (acetate-sulfate buffer) at a flow rate of 1.0 ml/min. Serum samples were injected by means of an autosampler with fixed volume of 2  $\mu$ l.

To determine the value for each fraction of albumin, i.e.,  $f(\text{HMA}) = [\text{HMA}/(\text{HMA} + \text{HNA})]$  and  $f(\text{HNA}) = [\text{HNA}/(\text{HMA} + \text{HNA})]$ , the obtained HPLC profiles were subjected to numerical curve fitting; each peak shape was approximated by a Gaussian function.

## Results and Discussion

HSA is known to be a mixture of HMA and HNA. Moreover, there are several kinds of HNA, i.e., HNA(Cys), HNA(Glut) and HNA(Oxi). A clear separation of three HSA fractions, i.e., HMA, [HNA(Cys) & HNA(Glut)] (tentatively called HNA-1 in this study) and HNA(Oxi) (called HNA-2) fractions was performed using our HPLC system in this study. Our previous studies have demonstrated that HSA redox state can be used as an index of biomarker of oxidative stress in the body (ERA et al., 1995; HAYASHI et al., 2000; IMAI et al., 2002; TOMIDA et al., 2003).

Representative HPLC profiles of HSA from a healthy subject (male, 45 years old) and a patient with both liver cirrhosis and esophageal cancer (male, 68 years old) are shown in Fig. 1A and B, respectively. There was a clear separation of HMA, HNA-1 and HNA-2 fractions. The obtained values for HMA, HNA-1 and HNA-2 fractions were 74.0, 24.3 and 1.7% (Fig. 1A) and 63.5, 34.8 and 1.7% (Fig. 1B), respectively. The  $f(\text{HMA})$  value of 74.0% for the healthy subject in this study was not different from a mean  $f(\text{HMA})$  value for normal subjects reported previously ( $73.2 \pm 2.3\%$ ) (IMAI et al., 2002). However, the  $f(\text{HMA})$  value of 63.5% for the patient with esophageal cancer was markedly lower than that for normal subjects, indicating that, in this patient with cancer, the defense mechanism against oxidative stress might have been greatly reduced. As there are no *in vivo* studies on the antioxidant effect of propolis, we first examined the influence of propolis ingestion on the redox state of HSA in a healthy subject (male, 45 years old). He took 12 tablets every day for 4 weeks. Surprisingly, his  $f(\text{HMA})$  value gradually increased from 74.0 (Fig. 1A) to 79.2%, and  $f(\text{HNA-1})$  value gradually decreased from 24.3 (Fig. 1A) to 19.9% over 4 weeks. This result suggested that his antioxidant level might have increased by propolis supplementation. Therefore, we examined the influence of propolis ingestion on the redox state of HSA in two patients with gastrointestinal cancer under the cancer therapy.

**Case 1:** A 56-year-old female patient with gastric cancer who had undergone curative gastrectomy. She complained of poor appetite probably caused by gastrectomy. She took 5 propolis tablets every day for 1 week, 6 tablets a day for 12 more weeks and continued to take 10 tablets a day for more 8 weeks. During the period of propolis supplementation, a sensation of poor appetite was improved and the  $f(\text{HMA})$  value was maintained between 73 - 79%, suggesting that propolis is a potentially effective substance for oxidative stress.

**Case 2:** A 68-year-old male patient with liver cirrhosis and esophageal cancer who had received radiation therapy. The  $f(\text{HMA})$  value before the radiation therapy without propolis ingestion was 66.6%. However, during radiation therapy for 2 weeks, he simultaneously took ten propolis tablets every day, and continued to take them for 4 more weeks. Values for  $f(\text{HMA})$  just before, immediately after, and 4 weeks after radiation therapy, were 63.5 (an HPLC profile is shown in Fig. 1B), 67.7 and 74.1%, respectively. Radiation therapy with  $^{60}\text{Co}$  radiation, even though it is the therapeutic dose for humans, has been known to produce a large number of oxygen radicals, and they are generally believed to be responsible for numerous findings of oxidative stress (DAVIES, 1987). However, our findings on  $f(\text{HMA})$  value in case 2 suggest that oxidative status due to radiation therapy might be attenuated by propolis supplementation. Furthermore, the gradual increase in  $f(\text{HMA})$  value indicated that the antioxidant properties of propolis might augment the total antioxidant capacity of patients with cancer.

We believe that these findings are strong support for the view that propolis plays a beneficial role as a supplemental antioxidant. To our knowledge, these are the first *in vivo* studies with analysis of oxidized and reduced albumin in cancer patients with severe oxidative stress and observation of the antioxidant effect of propolis. Of course, further studies with large numbers of cases are needed to confirm the validity of this finding, as well as to determine the clinical significance of the evidence reported in this paper.

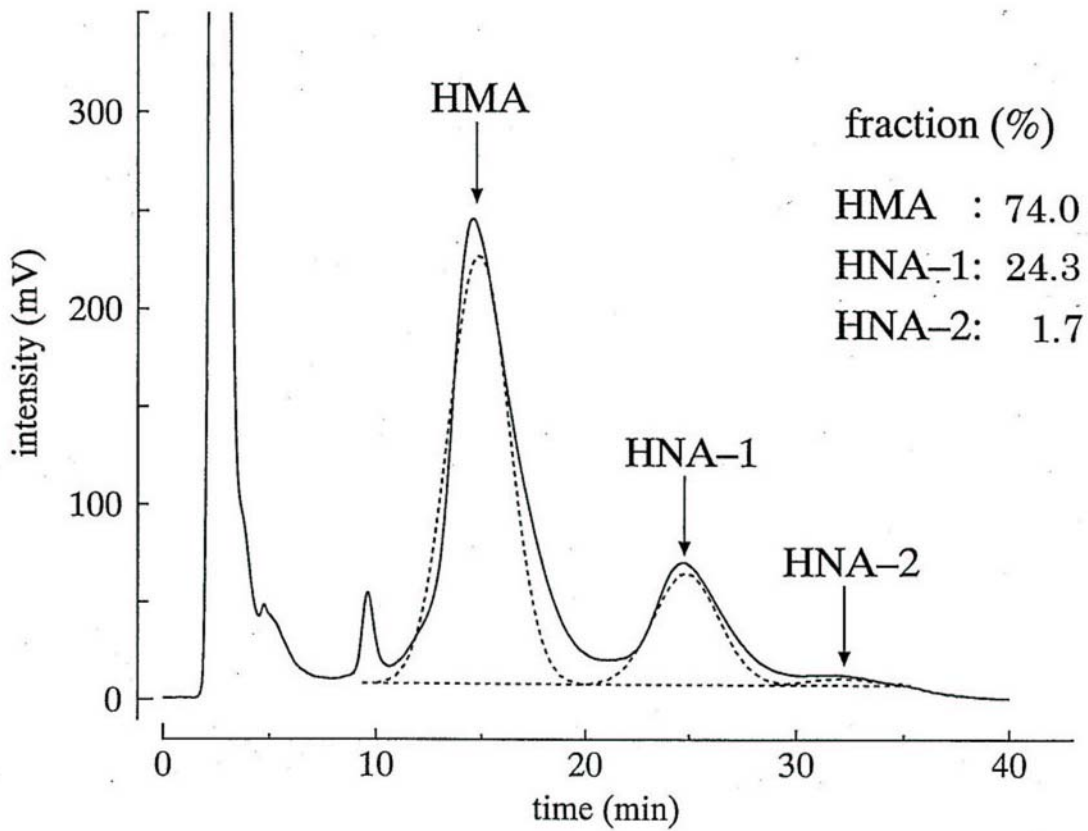


Fig.1A

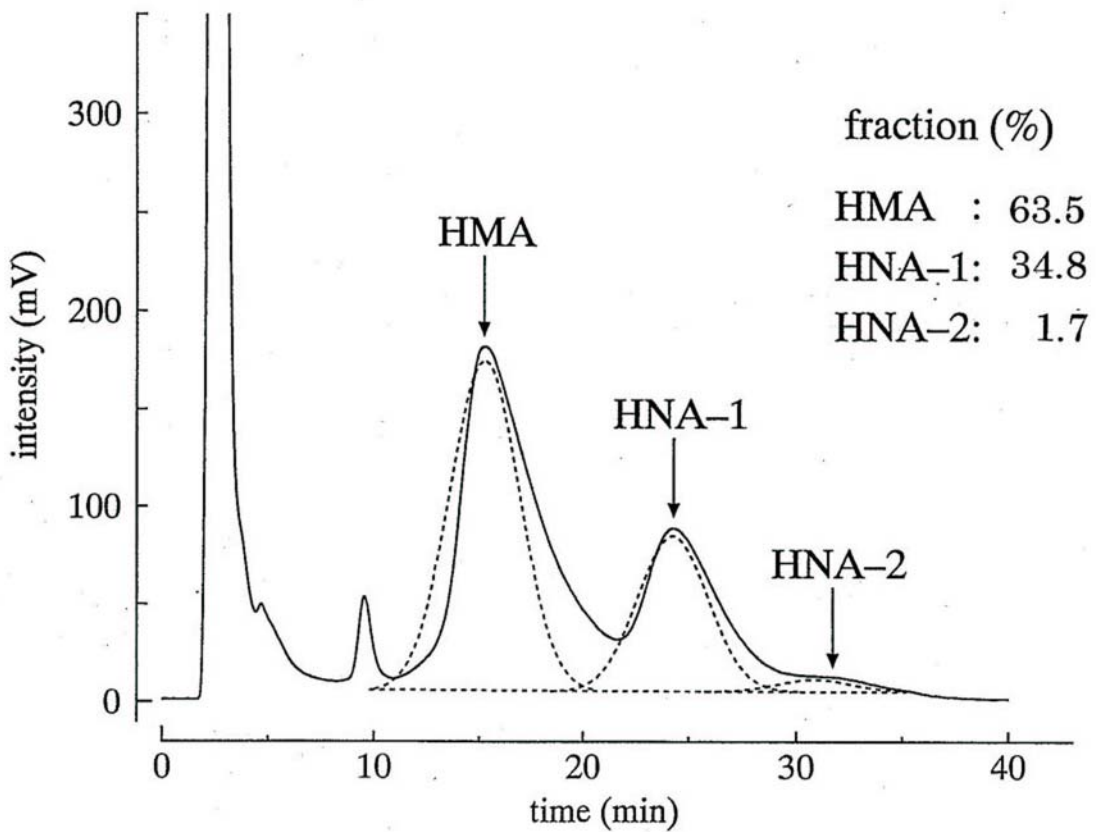


Fig.1B

Fig. 1 - Representative HPLC profiles of serum from (A) a healthy subject (male, 45 years old) and (B) a patient with liver cirrhosis and esophageal cancer (male, 68 years old) obtained by the HPLC system with fluorescence detection (excitation wavelength, 280 nm; emission wavelength, 340 nm). See the text for detailed elution conditions. The profiles are subjected to a numerical curve fitting (dashed line) and the obtained values for each fraction are indicated in the upper right of the figures.

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