Chemistry of Natural Compounds, Vol. 42, No. 4, 2006

INTERACTION OF WATER-SOLUBLE POLYSACCHARIDES OF *Limonium bicolor* WITH BSA

Lian-ru Zhang,[1] Xue-fen Yan,[1]
Zhong-hui Zheng,[1] and Guo-lin Zou[2*]

UDC 547.917

The interaction of the polysaccharide of Limonium bicolor *with BSA was measured at a neutral pH and an ionic strength of 0.15 M(NaCl), using SDS-PAGE and UV-Vis and fluorescence spectroscopy. The moving speed, the absorbance value, and the fluorescence of BSA are changed when glycan is present. The results have shown that the interaction is taken between glycan and BSA.*

Key words: glycan of *Limonium bicolor*, BSA, interaction

The function and bioactivity of polysaccharides has attracted much attention due to its importance in molecular recognition and cell conglutination [1] via the interaction between glycan with protein.

We have prepared a water–soluble polysaccharide (LP) with antitumor activity from the Chinese medicine herb *Limonium bicolor* [2], which is an endemic species of China and belongs to the family Plumbaginaceae [3]. The species belongs to a group of plants known as "China herb" that is used to heal bleeding, cancer, nephritis, and other weakness symptom. The cross-sectional plant *Limonium bicolor* grows in salty soil or grassland in China; the whole plant is widely used to enrich blood and treat hemostasis and is also used as a dry flower to decorate the environment by local folks [4]. In this paper, the interaction between the polysaccharide (LP) and BSA is discussed.

Figure 1 shows the SDS-PAGE of BSA and LP (shown below). From 1 to 6 the concentration of LP is increased. The migration speed of BSA is affected by LP; the lower the concentration of LP, the slower the moving speed of BSA in electrophoresis. The slow BSA migration can be explained by the interaction with LP, which changes the molecular mass of BSA. Much more studies have been done, as shown by the UV-Vis and fluorescence spectroscopy in Figs 2 and 3.

LP can decrease the absorbance of BSA and make the peak move to the red side. This is due to the structure of LP with the COO^- group, which can provide a negative charge to form a salt bond or hydrogen bond with the amino acid group of BSA. Otherwise, LP can also affect the fluorescence of BSA through the quenched emission spectrum. It is shown that the fluorescence of BSA can be quenched by drop of LP in the BSA and Tris-HCl buffer (pH 7.4) system. The interaction between LP and BSA can be explained according to the Stern-Volmer and Scatchard equations [5–6]:

$$F_0/F = 1 + K_{sv}[Q]$$

$$n[QnP]/[Q] = K \times n[Pt] - K \times n[QnP]$$

From the above, we can deduce the equation of fluorescence strength as a function of BSA and LP concentrations.

$$F_0/F = K[Qt]F_0/(F_0–F)-nK[Pt]$$

F_0: the fluorescence of BSA; Q: the concentration of LP; Pt: the concentration of BSA; n: the binding site. By plotting the curve of F_0/F versus c (LP) $F_0 / (F_0–F)$ and making a linear fit, the binding constant between BSA and LP can be calculated. Here K is 5.82×10^4 L/mol, and the relative coefficient is 0.9951, more than one binding site. We suggest that the binding may be due to the hydrogen bond or the hydrophobic interaction between the glyco residue of LP and the amino acid residue of BSA.

The experiments showed that the interaction between LP and BSA is responsible for the antitumor activity and other activities of LP.

1) College of Life Sciences, Xiamen University, Xiamen, 361005, Fujian, P. R. China, e-mail: zhanglianru@hotmail.com; 2) *College of Life Sciences, Wuhan University, Wuhan, 430072, Hubei, P. R. China, e-mail: zhanglianru@hotmail.com, tel: +86 592 8482781. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 317-318, July-August, 2006. Original article submitted August 1, 2005.

0009-3130/06/4204-0389

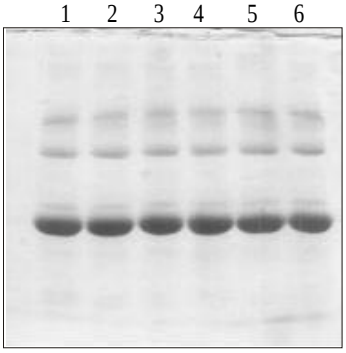

Fig. 1. The discontiguous SDS-PAGE of BSA and LP (*1*: 40 μLBSA; *2*: 40 μL BSA + 10 μLLP; *3*: 40 μLBSA + 20 μLLP; *4*: 40 μLBSA + 30 μLLP; *5*: 40 μLBSA+40 μL LP; *6*: 40 μLBSA+50 μLLP.

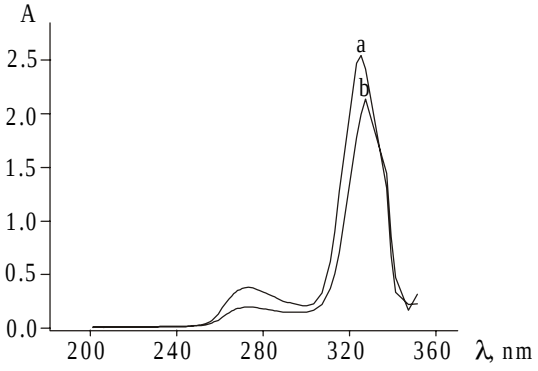

Fig. 2

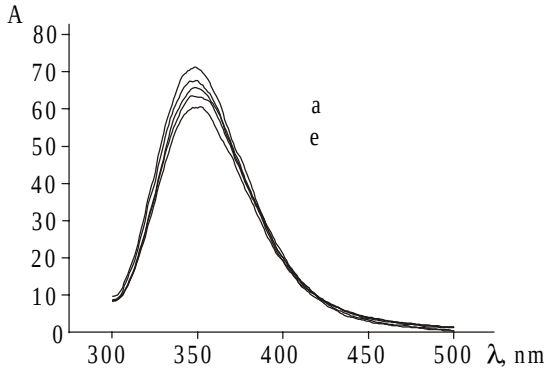

Fig. 3

Fig. 2. Absorbance spectrum of BSA. *a* – BSA, *b* – BSA + LP.

Fig. 3. Effect of LP on fluorescence spectrum of BSA a→e: *c*(LP)/*c*(BSA) = 0, 0.2, 0.4, 0.6, 0.8, 1.0

EXPERIMENTAL

Water-soluble LP was prepared according to [2]. The SDS-PAGE is discontiguous, the condensed glue is 3.7%, the separate glue is 8%, and the electrode buffer Tris-Gly (pH 8.3) includes 0.1% SDS; the electric current is 16 mA at the beginning, then 18 mA when the sample reaches the separate glue.

The absorbance was tested in TU-1800PC UV-Vis spectrophotometers. The concentration of LP is 0.1 mol/L, and that of BSA 0.1 mol/L, in 50 mmol/L Tris-HCl (pH 7.4), with NaCl (0.15 mol/L) to maintain the ion concentration.

The fluorescence was tested in an RF-5301 spectrophotometer; fast scan, narrow slot 3 nm. The test conditions are: BSA (1.0×10^{-6} mol/L) 3 mL in 1 cm test cup, then drop in LP (2 mg/mL) one by one, total volume of LP less than 100 μL.

REFERENCES

1. A. Varki, R. Cummings, and J. Esca, *Essentials of Glycobiology* (2004); http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books.
2. Zhang Lian-ru, Chen Ka-lin, Li Ni, and Zou Guo-lin, *Chem. J. Chin. Univ.*, **23**, 2394 (2004).
3. Lie-Chwen Lin and Cheng-Jen Chou, *Planta Med.*, **66**, 382 (2000).
4. Wang Bing-Wen, Zhu Rong, and Shen Si-Qing, *J. XI′AN Med. Univ.*, **15**, 59 (1994).
5. Chen Guo-Zhen, *The Method of Fluorescence Analysis*, Beijing: Science publishing Co., 122 (1990).
6. Huang Bo, Zou Guo-lin, and Yang Tian-ming, *Acta Chimica Sinica*, **60**, 1867 (2002).