ルブラトキシンBと血清アルブミンの相互作用

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Interaction of rubratoxin B with serum albumin

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Summary

The interaction of rubratoxin B (RB) with bovine serum albumin (BSA) was investigated. Circular dichroism spectroscopy shows the interaction of RB with BSA as conformational change of BSA. Fluorescence quenching study suggests that RB binds to BSA at a few sites. At low concentration of RB, RB firstly binds to BSA at any binding sites far from Trp residues. By increasing RB concentration, RB binds to BSA at any binding sites near Trp residues.

Key words : rubratoxin B, albumin, interaction, CD spectrum, fluorescence quenching

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Introduction

Rubratoxin B (RB) is a toxic secondary metabolite produced by certain Penicillium fungi[1]. Human rubratoxicosis was reported, indicating that RB can be a threat to human health[2]. Also, it was reported that RB shows hepatotoxicity, nephrotoxicity and spleentoxicity in several animals[3-7].

It has been unknown how RB is transported to liver and other organs after the ingestion of RB-contaminated foodstuffs. One possibility is that RB is transported by serum protein.

Serum albumin is the most abundant protein in plasma, and is one of the major soluble protein constituents of the circulatory system. It is known that serum albumin has many physiological functions. Among them, the most outstanding property of albumin is the ability to reversibly bind a large variety of compounds.

To date, the interaction of RB with serum albumin has not been investigated. In this paper, we report the state of interaction between RB and albumin *in vitro*. Because the knowledge of this interaction is thought to be very important in both clarification of RB dynamics in living systems and

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the treatment of rubratoxicosis.

Materials and Methods

Chemicals

Rubratoxin B (RB) and essentially fatty acid free (fraction V) bovine serum albumin (BSA) were obtained from Sigma-Aldrich (USA). Other chemicals were obtained from local commercial sources, and used without further purification.

Spectroscopy

BSA conformation was examined with and without RB conditions by circular dichroism (CD) spectroscopy. CD spectra were taken with JASCO J-720W spectropolarimeter (JASCO, Tokyo, Japan).

BSA was dissolved in 50 mM phosphate buffer (pH 7.4) at a concentration of 5 μM. RB concentrations ranged from 0 to 50 μM in BSA solution. Fluorescence spectra were taken with Shimadzu RF-5300PC spectrofluorometer (Shimadzu, Kyoto, Japan) using excitation of 280 nm and the emission range set between 280 and 450 nm. Fluorescence quenching analysis was carried out by measuring the fluorescence intensities at 355 nm.

Results and Discussion

Figure 1 shows CD spectra of BSA with and without RB. Solid line is the spectrum of BSA with RB (5 μM), and dashed line is that of BSA alone. Deviation between these spectra is observed at the vicinity of 220 nm. In the case of addition of RB to BSA, the width of CD spectrum slightly expanded. The shape of spectrum is explained by wave functions of both ground and excited states. When the change is occurred

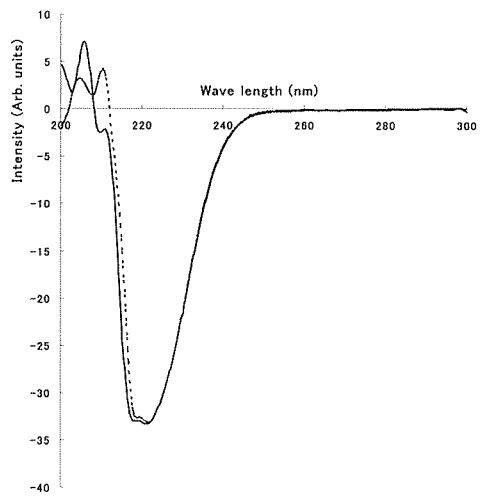


Fig. 1. Circular dichroism spectra of BSA.
Solid and dashed lines are the spectrum of BSA with RB (5 μM) and without RB, respectively.

in wave function in excited state, the phenomenon mentioned above can be observed. If the change in ground state is occurred, spectrum shape will be changed more drastically. One possible reason causing the change in excited state is considered to be the interaction of BSA and RB. By such interaction, the electronic state in excited state is influenced and resulted in the expansion of CD spectrum. Spectrum of this range represents the state of α-helix structure of BSA. The result shows that conformation of BSA, especially the arrangement of α-helix structure is affected by the interaction of RB with BSA.

Fluorescence spectra of BSA solution containing various concentration of RB were taken, and obtained spectra are shown in Fig. 2. The fluorescence arises from two tryptophan (Trp) residues in BSA protein. By analysis of the fluorescence quenching of Trp, the manner of interaction of RB with BSA can be examined[8]. RB concentrations of spectrum a, b, c, d and e are 0, 5, 12.5, 25 and 50 μM, respectively. By adding the equimolar (5 μM) of RB as BSA, fluorescence intensity increased, while, fluorescence quenching was occurred by increasing RB concentration.

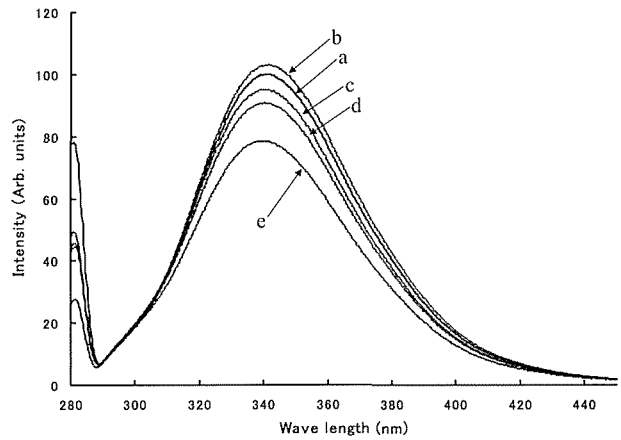
The fluorescence quenching data are analyzed by the Stern-Volmer equation (eq. 1)[9]

$$F_0 = F(1+K_{SV}Q) \text{ : (eq. 1)}$$

where F_0 and F are the relative fluorescence intensities of albumin without and with RB, respectively. K_{sv} is the Stern-Volmer quenching constant. Q is the concentration of RB. When RB binds to BSA at one site near the Trp residues, plot of fluorescence intensities at 355 nm in Fig. 2 can be approximated by eq. 1.

Figure 3 shows the result of Stern-Volmer analysis of Fig. 2. Solid line is approximation using eq. 1. It can be found that the fluorescence quenching data are not explained by Stern-Volmer analysis. The result means that RB binds to BSA at a few sites. It is likely that, at low concentration of RB, RB firstly binds to BSA at any binding sites far from Trp residues. In addition to far binding site from Trp residues, RB tends to bind to BSA at binding sites near the Trp residues according to RB concentration.

This study shows that RB interacts with BSA. As described in introduction, the possibility is also confirmed that RB is transported to liver and other organs by plasma proteins, after the ingestion of RB-contaminated foodstuffs.



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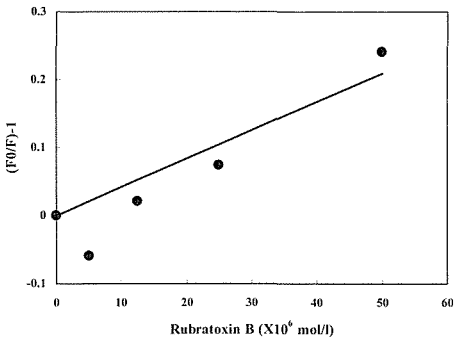

Fig. 3. Stern-Volmer plot of fluorescence data.
Solid line is approximation using eq. 1.

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ルブラトキシンBと血清アルブミンの相互作用について調べた．円二色性スペクトルを用いることで，ルブラトキシンBがアルブミンと相互作用することをアルブミンのコンフォメーション変化として捕らえることができた．また，アルブミン中のトリプトファン残基の蛍光消光より，ルブラトキシンBが低濃度では，アルブミンのトリプトファン残基より遠い位置に吸着され，ルブラトキシンB濃度の上昇に伴い，トリプトファン残基に近い位置にも吸着され始めることがわかった．

キーワード：ルブラトキシンB，アルブミン，相互作用，CDスペクトル，蛍光消光