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Spectroscopic analyses and studies on respective interaction of cyanuric acid and uric acid with bovine serum albumin and melamine

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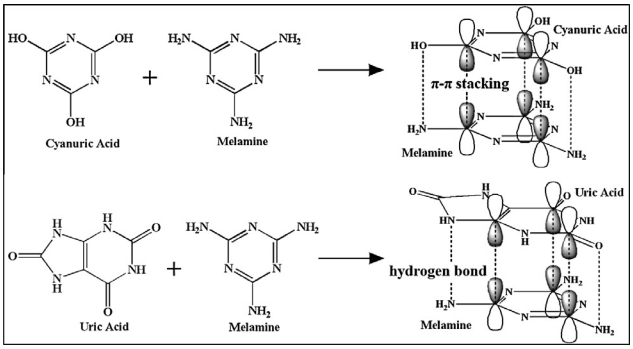
HIGHLIGHTS

- Interaction of CYA and UA with BSA and Mel were studied by spectroscopy.
- Binding sites of CYA and UA to BSA were confirmed by synchronous fluorescence.
- Interaction mechanism of CYA and UA with MEL was proposed.

GRAPHICAL ABSTRACT

Fluorescence spectra were used to study the interaction of cyanuric acid (CYA) and uric acid (UA) with BSA at different temperatures. The bimolecular quenching constant (K_q), apparent quenching constant (K_{sv}), effective binding constant (K_A) and corresponding dissociation constant (K_D), binding site number (n) and binding distance (r) were calculated using Stern–Volmer, Lineweaver–Burk and Double logarithm equations. The results show that both CYA and UA are able to bind to BSA, and the binding strength is BSA-CYA < BSA-UA. The interaction of CYA and UA with melamine (MEL) was also studied. The results will help us understand the formation of stones in the body after ingesting the MEL.



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ABSTRACT

In this work, the fluorescence quenching was used to study the interaction of cyanuric acid (CYA) and uric acid (UA) with bovine serum albumin (BSA) at two different temperatures (283 K and 310 K). The bimolecular quenching constant (K_q), apparent quenching constant (K_{sv}), effective binding constant (K_A) and corresponding dissociation constant (K_D), binding site number (n) and binding distance (r) were calculated by adopting Stern–Volmer, Lineweaver–Burk, Double logarithm and overlap integral equations. The results show that CYA and UA are both able to obviously bind to BSA, but the binding strength order is BSA + CYA < BSA + UA. And then, the interactions of CYA and UA with melamine (MEL) under the same conditions were also studied by using similar methods. The results indicates that both CYA and UA can bind together closely with melamine (MEL). It is wished that these research results would facilitate the understanding the formation of kidney stones and gout in the body after ingesting excess MEL.

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Introduction

Melamine (MEL) is a nitrogen heterocyclic triazine compound that is widely used as an industrial chemical in the production of plastic, coatings, and flame retardants [1]. In fact, the MEL is neither drugs nor food additives. Therefore, it is not allowed to add into any food. However, because of the higher nitrogen content, the MEL is illegally added into the pet food and milk to ostensibly increase the protein content by unscrupulous traders [2]. The upward momentum of illegal use of MEL has brought many serious problems, particularly, seriously damaged the health of the children. Cyanuric acid (CYA) can also be found in pet food as a co-contaminant, and it is added intentionally or perhaps is a by-product of melamine synthesis [3]. In addition, some microorganisms are able to metabolize MEL into CYA. Therefore, it can also be found in human intestines where the intestinal microflora live [4,5]. Normally, under low concentration condition the separate MEL and CYA do not display a very obvious toxicity to human [6–8]. However, when the MEL and CYA are ingested at the same time they will show extreme toxicity even cause the outbreak of acute renal failure [8–11]. Studies have demonstrated that the combination of MEL and CYA caused renal impairment through forming nearly insoluble renal crystals in different species [12]. Until now, research on the toxicity of the combination of MEL and CYA has mainly focused on the lesions to the kidney, including formation of crystals in renal tubules, necrosis in the tubular epithelia and renal interstitial edema [13,14].

Uric acid (UA) as a structural analogue of MEL is a human cell metabolism and food ribonucleic acid products of purine metabolism, and sustained high blood UA concentrations can lead to gout easily [15]. And some studies have found when the re-uptake of MEL happens in body, the UA also interacts with MEL to form stable complexes. MEL combines with CYA and UA to form stable crystals that have been known to be nephrotoxic [16]. That is, MEL, forming such crystals, can aggravate the formation of renal stones. Because of the structure particularity, CYA and UA have special effect on various biomolecules [17–19]. Similarly, the MEL should also interact with biomolecules, such as proteins and its analogues, and then could be transported and display a variety of biological activity. Therefore, in order to understand the forming process of MEL renal stones and influence of CYA and UA it is necessary to study the interaction among them.

As well known, as one of the most abundant carrier proteins, the serum albumin is indispensable in the transport and disposition of endogenous and exogenous ligands present in blood. In addition, it also has many physiological functions. In recent years, the bovine serum albumin (BSA) was often chosen as a target protein molecule because of its low cost, ready availability and unusual ligand-binding properties [20–23]. And what's more, the whole structures of BSA molecule are similar to human serum albumin (HSA) molecule in 70%. The results of all the studies are considered to be consistent with the facts using HSA, so the BSA was generally used as a model of biological macromolecules in previous works [24].

So far, the interaction of CYA and UA with BSA as well as the interaction of CYA and UA with MEL has not been studied in detail. In this paper, the interaction of BSA with CYA and UA at different temperatures were studied by means of fluorescence spectra. The relative interaction strength and binding mode were also examined by synchronous fluorescence spectroscopy. According to the fluorescence quenching the various parameters were calculated and the corresponding quenching mechanisms were also confirmed. In addition, in order to judge the binding of MEL with CYA and UA, the interaction of MEL with CYA and UA were also studied. It is hoped that this work could offer some valuable information in the field of researching toxicity and harm of MEL.

Experimental section

Materials

Melamine (MEL, analytical purity, Sinopharm Chemical Reagent Co., Ltd., China), Cyanuric acid (CYA, analytical purity, Sinopharm Chemical Reagent Co., Ltd., China) and Uric acid (UA, analytical purity, Sinopharm Chemical Reagent Co., Ltd., China) were purchased and used directly without further purification. Bovine serum albumin (BSA, biochemical reagent, Abxing Biotechnological Company, China) was acted as a model protein to study the interaction activities of Cyanuric acid (CYA) and Uric acid (UA). Tris (hydroxylmethyl) aminomethane (Tris), NaCl and HCl (analytical purity, Shenyang Chemical Reagent Plant, China) were used to prepare the Tris-HCl–NaCl (pH = 7.4 and [Tris-HCl] = [NaCl] = 50 mmol L^{-1}) buffer solution and to adjust the solution acidity and maintain the ionic strength. The other chemical reagents were all analytical reagent grade, and double distilled water was used for preparing solution.

BSA and MEL stock solutions (2.00×10^{-5} mol L^{-1}) were prepared by dissolving BSA and MEL, respectively, with Tris-HCl–NaCl (pH = 7.4 and [Tris-HCl] = [NaCl] = 50 mmol L^{-1}) buffer solution. The BSA solutions were stored in refrigerator at 0–4 °C. The CYA and UA stock solutions (5.00×10^{-5} mol L^{-1}) were obtained by dissolving CYA and UA with Tris-HCl–NaCl (pH = 7.4 and [Tris-HCl] = [NaCl] = 50 mmol L^{-1}) buffer solution. The molecular structures of melamine (MEL), cyanuric acid (CYA) and uric acid (UA) are presented in Fig. 1.

Apparatus and instruments

The fluorescence measurements were performed on a fluorophotometer (Cary 300, Varian Company, USA) and the UV–vis absorption spectra were recorded with an UV–vis spectrophotometer (Cary 50, Varian Company, USA). The two temperatures were dominated at 10.0 ± 0.2 °C and 37.0 ± 0.2 °C in the whole experiment.

Measurement of binding parameters

For each of the CYA and UA, the binding parameters with BSA molecules were measured by fluorescence spectroscopy. CYA and UA with BSA stock solutions were prepared using Tris-HCl–NaCl buffer solution (pH = 7.40 and [Tris-HCl] = [NaCl] = 0.05 mol L^{-1}). To a 25.00 mL volumetric flask, 12.50 mL BSA stock solution (2.00×10^{-5} mol L^{-1}) and appropriate volume of CYA or UA stock solution (5.00×10^{-5} mol L^{-1}) were added and then diluted to the mark with Tris-HCl–NaCl buffer solution (pH = 7.40 and [Tris-HCl] = [NaCl] = 0.05 mol L^{-1}). The final BSA concentration was 1.00×10^{-5} mol L^{-1}, and the CYA or UA concentrations were varied from 0.00×10^{-5} mol L^{-1} to 2.50×10^{-5} mol L^{-1} at 0.50×10^{-5} mol L^{-1} intervals. The fluorescence spectra of BSA solutions along with the increase of CYA or UA concentrations were recorded as shown in Fig. 2 in the wavelength of 200–500 nm with excited wavelength at 278 nm and 5.0 nm/5.0 nm slit widths. All test solutions were incubated for 10 min before measurement, and then the curves of fluorescence quenching spectra were obtained at 10.00 ± 0.02 °C (a-1, b-1 and c-1) and 37.00 ± 0.02 °C (a-2, b-2 and c-2), respectively. The maximal intensities of the intrinsic fluorescence of BSA solutions were recorded at 348 nm for the calculation of quenching parameters. The corresponding results were shown in Fig. 3 and Table 1, respectively. The binding distances were also calculated according to Föster's nonradiative energy transfer theory (FRET). The spectral overlap of fluorescence emission of BSA solution and UV–vis absorption of CYA or UA solutions were