

FLUORESCENCE SPECTROSCOPY STUDY OF THE INTERACTION BETWEEN OCHRATOXIN A AND HUMAN SERUM ALBUMIN IN THE PRESENCE OF FLURBIPROFEN

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Many natural toxins exist in the human environment, which are potentially pathogenic. One of the major groups are mycotoxins produced by some molds. This group includes ochratoxin A (OTA), produced mainly by the strain *Aspergillus ochraceus*, which commonly occurs in nature and is responsible for rotting of food articles (1, 2). OTA is responsible for the occurrence of many pathologies in the human organism. It shows a strong nephrotoxic, teratogenic, genotoxic and mutagenic effect (3, 4). As far as the chemical structure is concerned, OTA is a compound built of the amino acid α -phenylalanine connected with an isocoumarin derivative by a peptide bond (2).

Human serum albumin HSA is the main protein of human blood plasma and accounts for about 60% of the whole plasma protein. Due to the high concentration in blood serum and unspecificity of the bond, albumin plays the main role in binding and

distributing endogenous and exogenous substances in the organism. The most important binding sites are sites I and II (Sudlow site I and Sudlow site II). Substances strongly bound to plasma proteins to a lesser extent penetrate into the organs (5–12).

Flurbiprofen belongs to propionic acid derivatives, the most frequently represented group of non-steroidal antiinflammatory drugs. Its half-life period is about 4–5 h. The metabolism of flurbiprofen occurs mainly in the liver and its metabolites are excreted by kidneys with urine (13).

MATERIALS AND METHODS

Ochratoxin (OTA), albumin (HSA) and flurbiprofen samples were acquired from Sigma-Aldrich. Samples of OTA with a concentration of 1 μ M dissolved in PBS solution were used for the analysis. PBS buffer with pH 7.4 was obtained by dissolving 8.0066 g 137 mM NaCl; 0.2236 g 3 mM KCl; 1.1357 g 8 mM Na₂HPO₄ and 0.1361 g 1 mM KH₂PO₄ in 1 liter of distilled water. Then, appropriate amounts of 0.1 M KOH or 0.1 M KCl were added to obtain the desired pH. PBS buffers were stored under cooling conditions. Before each new analysis, PBS pH was checked and the buffer was heated to 37°C. High resolution optic spectrometer of HR4000 (Ocean Optics)

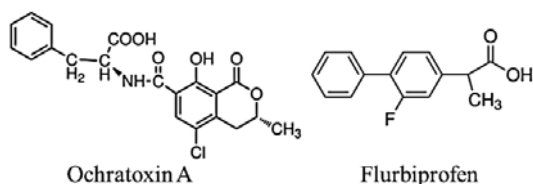


Figure 1. Structural formulas of ochratoxin A and flurbiprofen (2)

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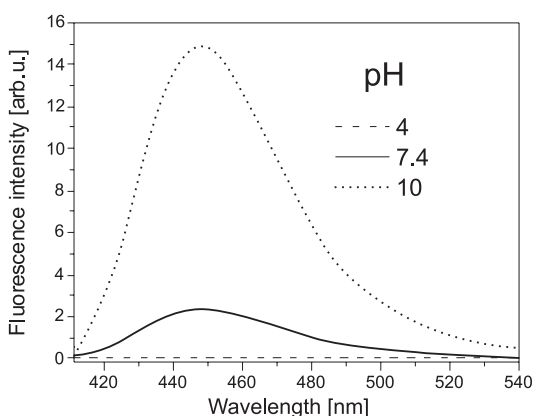


Figure 2. OTA fluorescence emission spectra in PBS at different pH. OTA concentration = 1 μ M

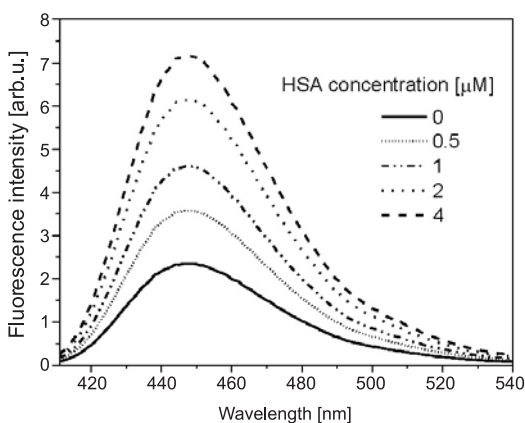


Figure 3. OTA fluorescence emission spectra in different concentration of HSA at pH 7.4

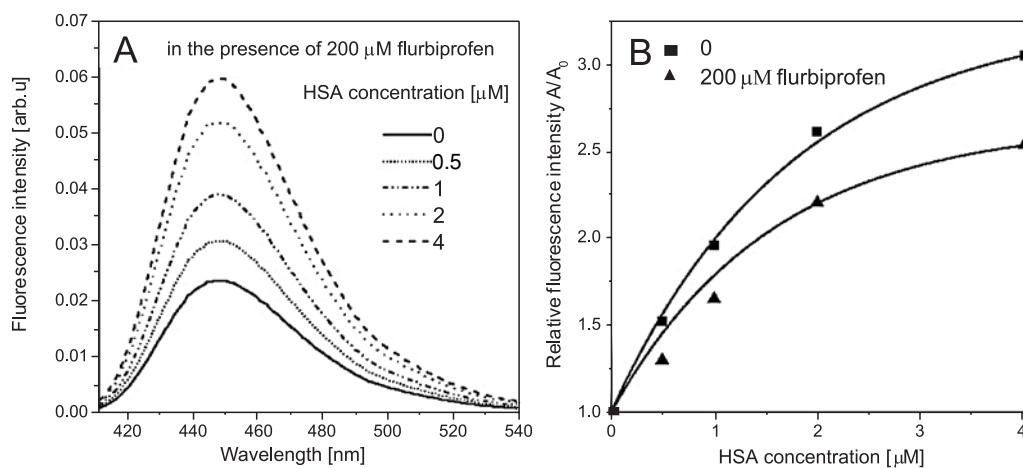


Figure 4. (A) OTA fluorescence emission spectra in HSA suspension with different concentration in the presence of flurbiprofen with a concentration of 200 μ M at pH 7.4. (B) Relative increase in OTA fluorescence intensity at 448 nm depending on HSA concentration in the absence (0) and presence (200 μ M) of flurbiprofen

was used to register fluorescence emission spectra. Radiation with a wavelength of 395 nm was used to excite OTA (the source was a electroluminescence diode).

RESULTS AND DISCUSSION

Figure 2 presents the fluorescence emission spectra of OTA dissolved in PBS buffer with different pH values. The diagram shows that OTA in pH 4 does not fluoresce. This results from the fact that in acid pH OTA occurs only in the monoanionic form. This form of ochratoxin does not adsorb light with a wavelength of 395 nm. The intensity of OTA fluorescence increases along with the increase in pH, which is the consequence of passing from the monoanionic to dianionic form (5, 10) Quite large fluorescence can already be observed at physiological pH (7.4). This is caused by the occurrence of both monoanionic and dianionic forms at this pH. Fluorescence with a large intensity is observed at alkaline pH (pH = 10). This results from the occurrence of ochratoxin only in the dianionic form, which strongly fluoresces.

In order to examine the effect of OTA on albumin, OTA fluorescence spectra with a concentration of 1 μ M were registered in the presence of albumin with different concentrations from 0 to 4 μ M at physiological pH (Fig. 3). The maximum of OTA fluorescence emission spectrum in PBS buffer with a pH of 7.4 occurs at the wavelength 448 nm. Along with increasing HSA concentration, an increase in the maximum of OTA fluorescence intensity is observed. This is related to the fact that the monoan-

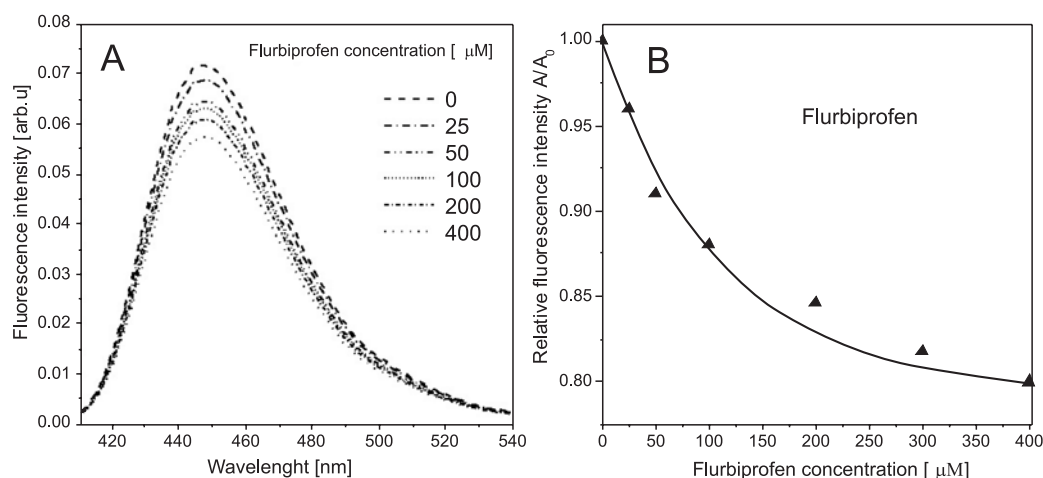


Figure 5. (A) OTA fluorescence emission spectra in HSA suspension with a concentration of 3 μM depending on the flurbiprofen concentration. (B) Relative OTA fluorescence at 448 nm in the function of flurbiprofen concentration at the constant HSA concentration (3 μM)

ionic form passes to the dianionic form by binding to protein (5, 10). Thus, fluorescence intensity depends on the amount of OTA particles bound to HSA.

Figure 4A presents the OTA fluorescence emission spectrum at pH 7.4 depending on HSA concentration after the addition of flurbiprofen with a concentration of 200 μM . Along with increasing HSA concentration, an increase in fluorescence intensity at the wavelength 448 nm is observed, but it is considerably smaller than in the case presented in Figure 3. Figure 4B presents a relative increase in OTA fluorescence intensity at the wavelength 448 nm in the function of HSA concentration, based on fluorescence emission spectra from Figures 3 and 4A. It can be concluded that flurbiprofen, which is characterized by a high affinity to the same binding sites in albumin as ochratoxin A, causes its displacement from protein. Free ochratoxin, in turn, returns to the monoanionic form, which does not fluoresce.

Fig. 5A presents OTA fluorescence emission spectra at pH 7.4 depending on flurbiprofen concentration at the constant HSA concentration equal to 3 μM . After the addition of flurbiprofen to the OTA-HSA solution, a decrease in OTA fluorescence intensity is observed. This results from displacement of OTA from protein and return of OTA to the monoanionic form. A decrease in fluorescence intensity is directly proportional to the concentration of added flurbiprofen. Figure 5B presents how a relative fluorescence of the OTA-HSA complex changes at a wavelength of 448 nm in the function

of flurbiprofen concentration. As the figure shows, the binding of OTA to albumins can be competitively replaced with some non-steroidal antiinflammatory drugs, such as flurbiprofen. This will accelerate the metabolism of OTA and contribute to faster excretion of this toxin from the organism. On the other hand, instead of chronic toxicity, the use of flurbiprofen may contribute to the occurrence of acute toxicity shortly after exposure to the toxin. Therefore, the use of this medicine must be connected with evaluation of the patient's condition and requires great caution.

CONCLUSION

The present study proved that ochratoxin A occurs in different ionic forms depending on the pH of the environment. At acidic pH it occurs in the monoanionic form, at alkaline pH in the dianionic form, whereas at physiological pH the occurrence of both forms is observed. Experimental results show that apart from the pH of the environment, the presence of HSA has also the essential effect on the ionic form of OTA. Since only the dianionic form of ochratoxin can be bound to albumin, the monoanionic form is transformed into the dianionic form under the influence of protein. Changing the ionic form of ochratoxin is connected with opening of its lactone ring. Results of the study show that flurbiprofen to a considerable extent affects a reduction in binding of ochratoxin to albumin. This non-steroidal antiinflammatory drug binds with albumin and is competitive for OTA binding sites.

Consequently, it shortens the time of ochratoxin presence in the vascular bed. On the one hand, thanks to binding OTA by plasma proteins, the toxin penetration into the target tissues of the organism is delayed and reduced, while on the other, it prolongs the presence of this toxin in the vascular bed. Flurbiprofen and its possibly protective effect against the toxic effect of OTA suggests further studies of its application as an antidote.

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