

## Effect of bovine serum albumin on the UV spectra of 5-aminoorotic acid and its complex with Pr(III) in K, Na- phosphate buffer of pH 7.45

V. Atanasova<sup>1</sup>, C. Costanzo<sup>2</sup>, L. Todorov<sup>1</sup>, M. Valcheva-Traykova<sup>2</sup>, I. Kostova<sup>1\*</sup>

1- Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria

2- Department of Medical Physics and Biophysics, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria;

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Interactions with blood serum albumin are essential for the pharmacokinetics and metabolism of pharmaceuticals. 5-Aminoorotic acid (HAOA) and its complex with Pr(III) (PrAOA) are promising anticancer agents with antioxidant activity, latter still under intensive investigation. The interaction of HAOA and PrAOA with blood serum albumin has not been investigated yet.

The effects of 0.01, 0.1 and 1 mg/ml of bovine serum albumin (BSA) on the UV spectra of  $10^{-4}$  M PrAOA and  $3.10^{-4}$  M HAOA were monitored. UV-VIS spectra were recorded on Shimadzu 1601 spectrophotometer, at very low speed, using quartz cuvette.

The presence of BSA significantly and in a different manner altered the UV spectra of both compounds, the effect being as strong as high the BSA concentration was. The >N-H, -NH<sub>2</sub>, and C=O characteristic bands were mostly influenced by the interactions of the molecules with BSA.

Spectra of PrAOA and HAOA in the presence of BSA revealed a possible difference in attachment of these compounds to albumin. Involvement of the >N-H, -NH<sub>2</sub>, and >C=O groups of PrAOA in interactions with BSA were registered. PrAOA did not dissociate during attachment to BSA.

The attachment of PrAOA to BSA possibly proceeded with participation of the ligands of the intact complex. This would allow the albumin to carry the intact PrAOA in the blood stream, preserving this way the environment from the prooxidant action of the Pr(III) ion.

**Key words:** 5-aminoorotic acid, Pr(III), UV-spectroscopy, bovine serum albumin, complex Pr(III)-5-aminoorotate.

### INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in the blood plasma serving as a carrier of many endogenous and exogenous compounds [1,2]. The bioavailability of many biologically active species (ions, complexes, molecules) is related to their successive binding to HSA [3]. The binding constants of the ligands to serum albumin determine their distribution, free concentration, excretion and metabolic transformations in the body [4]. Bovine serum albumin, BSA, is widely used as a model of HSA [4,5]. BSA is easily available, affordable and water soluble, latter facilitating investigations in aqueous solutions.

HAOA is an amino-derivative of the biologically active orotic acid, HOA (Vitamin B<sub>13</sub>), latter being intermediate in the biosynthesis of DNA and RNA. Theoretical studies of these compounds and their complexes with some Ln(III) ions were performed [6-10] and significant cytotoxic activity of some Ln(III) complexes in different human cell lines have been detected, along with ROS scavenging, cell protection, cytoskeletal stabilization and immunologic enhancement [11-20].

The formation and elimination of free radicals determine the level of oxidative stress and are associated with the health status of a living system. The radicals scavenging activity of the Ln(III) complexes [10,13,15,17-19] might be associated with *in vitro* and potential *in vivo* antioxidant activity of the ligands. The oxidative behavior of these complexes resulted from the radicals scavenging properties of the ligands and the radicals production of the free Ln(III) ions. The oxidative behavior of the Ln(III) complexes with biologically active organic ligands was related to effects of the chelator on the organic ligands as well as with the stability of the complexes in the environment.

Recently Pr(III) complex of HAOA (PrAOA) was synthesized and its involvement as antioxidant in the OH<sup>-</sup> induced oxidative stress in the presence of Fe(II)/EDTA/H<sub>2</sub>O<sub>2</sub> model system in rat blood serum was reported [21]. The radicals scavenging activities suggested antioxidant activity of HAOA both as a free compound and as a ligand, and prooxidant activity of Pr(III) ion. The UV spectra showed solvent effect on the dissolved molecules. The radicals scavenging activity of HAOA in homeostatic PBS was related with partial dissociation and shift in the energy of some

\* To whom all correspondence should be sent:  
E-mail: irenakostova@yahoo.com

electron transitions of the intact molecules. PrAOA did not dissociate in homeostatic PBS. The weaker *in vitro* free radicals scavenging activity of PrAOA than this of HAOA was associated with effect of Pr(III) on the partial charge distribution and dipole moment of the ligands.

The interactions with blood serum albumin of HAOA and PrAOA are not elucidated yet, but they are essential for the transport in the blood compartment and the biological activities of the compounds. In the present work these interactions were investigated by observing the relative changes in the UV spectra of HAOA and PrAOA in the presence of BSA.

### MATERIALS AND METHODS

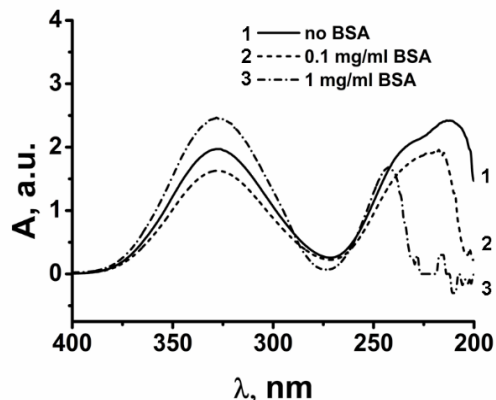
All chemicals were of highest grade, and bi-distilled water was used for the solutions. The standard solutions were: 50 mM K,Na-PBS with pH 7.45 (PBS), 10 mg/ml BSA,  $10^{-3}$  M HAOA and  $10^{-3}$  M PrAOA dissolved in PBS. The effects of 0.01, 0.1 and 1 mg/ml BSA in PBS on the solutions containing  $10^{-4}$  M PrAOA and  $3 \cdot 10^{-4}$  M HAOA were monitored by scanning UV spectra within 400 and 200 nm. The HAOA concentration was chosen to correspond to the content of AOA<sup>-</sup> ligands in the  $10^{-4}$  M PrAOA. UV-VIS spectra were recorded on Shimadzu 1601 spectrophotometer, at very low speed (step of 0.5 nm), using 2 ml quartz cuvette. The instrumental errors were eliminated by recording the spectrum of the solvent against solvent. The experimental errors in wavelength positions ( $\pm 2$  nm) and absorbances ( $\pm 0.001$  a.u.) of the spectra were estimated by recording each spectrum 3 times. The spectra of the BSA solutions were subtracted from the spectra of the compounds dissolved in these solutions. Data were collected using the software of the apparatus, and then transferred in PC and spectra were resolved using ORIGIN program package. Only components of Gauss deconvolution, which resulted in theoretical spectrum which reproduced the experimental spectrum with  $R^2 \approx 0.99$  were discussed. The UV spectra were resolved using literature data [6,7,10,21-23].

### RESULTS

The effect of 0.1 mg/ml BSA on the spectra of HAOA and PrAOA was under the instrumental errors limits and was not discussed. The UV spectra of  $3 \cdot 10^{-4}$  M HAOA in absence of BSA and in the presence of 0.1 mg/ml and 1 mg/ml BSA were presented in Fig. 1.

By bands positions spectrum 1 was similar to this observed in [21] for HAOA dissolved in PBS. Spectra in Figure 1 indicated that the UV spectrum

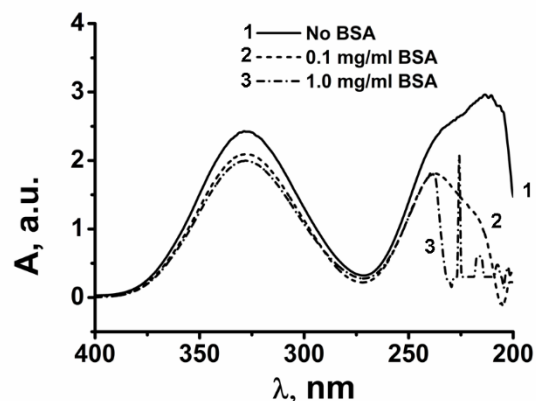
of HAOA depended on the concentrations of BSA in the solution. In agreement with [21], the Gauss deconvolution of the UV spectrum 1 in Fig.1 showed components characteristic for ionized (183 and 341 nm) and non-dissociated HAOA (321, 227 and 210 nm).



**Fig. 1.** UV spectra of HAOA: alone (1), in the presence of 0.1 mg/ml BSA (2) and in the presence of 1 mg/ml BSA (3).

In the presence of 1 mg/ml BSA most of the intensive bands in the region 300-200 nm disappeared, and only one prominent band at 245 nm was observed. Latter can be associated with characteristic band for the conjugated double bond system in HAOA (around 227 nm) shifted to the higher wavelength due to decreased energy. The characteristic band for the  $\pi \rightarrow \pi^*$  transitions in the aryl ring did not change in position, but increased in intensity, which might be related with changed dipole moment. The characteristic bands in spectrum 2 in Fig.1 showed relative decrease in intensities below 230 nm, but the shape of the spectrum was similar to spectrum 1 too.

The UV spectra of  $10^{-4}$  M PrAOA in PBS as well as in solutions containing 0.1 and 1 mg/ml BSA were presented in Fig. 2.



**Fig. 2.** UV spectra of PrAOA: alone (1), in the presence of 0.1 mg/ml BSA (2) and in the presence of 1 mg/ml BSA (3).

In agreement with [21], spectrum 1 in Fig. 2 showed characteristic bands for non-dissociated PrAOA complex (Gauss components at 327, 234 and 208 nm). Spectrum 2 in Fig.2 indicated a decrease in intensity of the bands below 300 nm and shift of the maximum in this region to 240 nm. In presence of 1 mg/ml BSA the UV spectrum of the complex drastically changed between 300 and 200 nm (Fig.2, spectrum 3). Along with the band at 240 nm, a new, very sharp maximum appeared at 226 nm. This band might result from vibrations of -NH<sub>2</sub> groups of the PrAOA in positions inaccessible for hydrogen bonding. The band at 240 nm in spectra 2 and 3 in Fig. 1 were of same intensity and may be associated with the conjugated double bonds in the aryl ring of the complex.

### DISCUSSION

Spectra 1 in Figures 1 and 2 were identical by shape and band positions with those reported in [21] for HAOA and PrAOA dissolved in PBS with physiological pH. As in [21], the PrAOA dissolved in PBS was not dissociated. The shapes and band positions of spectra 2 in figures 1 and 2 indicated that some HAOA and PrAOA interacted with the albumin in the presence of 0.1 mg/ml BSA, while some of them were still affected only by the solvent. Evidently concentration of 0.1 mg/ml BSA was not enough high to result in BSA interactions with all HAOA or PrAOA in the solution. Spectra 3 in Figures 1 and 2 suggested significant effect of BSA on the compounds investigated. Comparison between spectrum 3 in Fig. 1 with spectrum 3 in Fig. 2 indicated different effect of 1 mg/ml BSA on HAOA than this on PrAOA. The similarity in positions of the characteristic band at 245-240 nm in spectra 3, Fig. 1 and 2 indicated a decreased energy of the component corresponding to the conjugated double-bond systems, probably due to increased conjugation resulting from adsorption on BSA. The relative difference between spectra 3 seen in the two figures indicated that the interactions of HAOA and PrAOA with BSA proceeded with participation of individual -NH<sub>2</sub>, >C=O, and probably ->NH groups and resulted in elongation of the conjugated double-bond systems. The band at 228 nm might be assigned to -NH<sub>2</sub> group in the HAOA and AOA<sup>-</sup> ligand. In the spectrum 3 in Fig.1 it was very low intensive and sharp, while in spectrum 3, Fig. 2 this band was sharp and very intensive. The difference between spectra 3 in Fig.1 and 2 indicated different orientation of HAOA adsorbed on BSA than this of PrAOA. It may be proposed that more of HAOA participated in the interaction of this molecule with

BSA involving individual -NH<sub>2</sub> group, and very small amount of molecules were oriented with -NH<sub>2</sub> groups inaccessible for hydrogen bonding. The interaction of PrAOA with BSA might proceed with dominating orientation of the complex resulting in inaccessibility of -NH<sub>2</sub> to hydrogen bonding. The same position and relatively small difference in absorption of the characteristic band for the  $\pi \rightarrow \pi^*$  transitions in the aryl ring of the 5-aminoorotic acid (327 nm) in spectra 1, 2 and 3 in Figure 2 showed that BSA-PrAOA interaction proceeded without decomposition of the complex indicating its stability.

On the base of this investigation it may be proposed that HAOA and PrAOA interacted with BSA in different manners. The interaction of PrAOA with BSA proceeded with participation of the non-dissociated ligands of the complex. The predominant orientation of the complex resulted in inaccessibility of the -NH<sub>2</sub> group to hydrogen bonding. The fact that PrAOA complex was fixed on BSA without being decomposed is very important for the successful transportation of the complex with the blood stream, without prooxidant action of individual Pr(III) ions in the blood compartment. This will allow the complex to access the target zone (cancerous tumor), where PrAOA is expected to decompose and exert its anticancer action.

### CONCLUSIONS

1. Both 5-aminoorotic acid and its Pr(III) complex interact with BSA in physiological pH.
2. The interaction of Pr(III) complex of 5-aminoorotic acid with BSA is different than this of the ligand alone.
3. The Pr(III) complex of 5-aminoorotic acid was fixed on the BSA in a non-dissociated state, which might facilitate the transport of the intact complex in the blood stream, preserving the blood compartment from the prooxidant effect of the individual Pr(III) ions.

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