Chinese Chemical Letters Vol. 11, No. 3, pp. 247–250, 2000

Interaction of Nicotine and Bovine Serum Albumin

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Abstract: The binding of nicotine to bovine serum albumin (BSA) was studied by UV absorption, fluorescence, and ^{1}H NMR methods. With the addition of nicotine, the absorption band of BSA at about 210 nm decreased gradually, moved to longer wavelengths, and narrowed. BSA fluorescence of tryptophan residue was quenched by nicotine. The ^{1}H NMR peaks of nicotine moved to downfield by the addition of BSA. The experimental results showed that nicotine was capable of binding with BSA to form a 1:1 complex. BSA's high selectivity for nicotine binding suggests a unique role for this protein in the detoxification and/or transport of nicotine.

Keywords: Nicotine, BSA, UV absorption, fluorescence, ^{1}H NMR.

Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine, is a major alkaloid in tobacco products, typically composing 1-2% weight of tobacco. So far, there are a great deal of papers reporting the effect of nicotine on various biological tissues of animals and humans. The pharmacological effect of nicotine is a dominant addiction factor for smoking. Since a very large population is frequently exposed to nicotine through the mainstream and/or sidestream of smoking inhalation, the interaction of nicotine with biological tissues is a significant subject for study. Li *et al*[1]. first identified DNA adduction with nicotine *in vivo* by accelerator mass spectrometry (AMS), Wu *et al*[2]. also measured the mouse hepatic histone adduction with nicotine *in vivo*, demonstrating a good linear response of the number of adducts to exposed nicotine dose. The gene-regulatory functions of histone have been firmly discovered, so the adduction mentioned-above has shown the potential genotoxicity of nicotine.

Adduct formation between serum albumin and potential carcinogenic nicotine has not been reported previously. This protein could be of particular interest for a variety of reasons. Because of its important role in the binding and transport of xenobiotics and endogenous compounds, much has been learned about the mechanisms of interaction with different substrates[3]. For several species, including rats, bovine and humans, the complete amino acid sequence is known. Finally, serum albumin is a readily accessible protein that can be obtained routinely and without trauma.

Experimental

UV-visible absorption spectra were recorded in a Shimadzu Model UV 240

spectrophotometer with a slit width of 2 nm, using a 10-mm cell at room temperature. Fluorescence measurements were performed in a Shimadzu Model RF540 spectrofluorometer at 16 °C. The excitation wavelength was 285 nm, the emission wavelength window was 300-500 nm. ^{1}H NMR were obtained at 400 MHz in a Bruker AXL 400 spectrometer under the following conditions: in a phosphate buffer (20 mM phosphate, 0.0001 M EDTA, 0.1 M NaCl, and pD 7.4), prepared by D_2O (99.99%, and the temperature of the sample tube was maintained at 24°C. The chemical shifts in parts permillion(ppm) were referred with respect to that of water.

Results and Discussion

Fluorescence of BSA

The fluorescence emission spectrum of BSA exhibits the typical emission band of tryptophan, at 345 nm, by excitation with 285 nm light. The fluorescence quenching of BSA by nicotine as shown in **Figure 1** was remarkable, and the maximal fluorescence (λ_{max}) of BSA was moved to shorter wavelengths (blue shifts). According to reference[4], in the case of bovine albumin binding of 1 mole of the ligand results in a quenching of the tryptophan fluorescence, we obtain an evidence for the formation of a 1:1 BSA-nicotine complex. That is compatible with UV absorption spectroscopic studies.

UV absorbing spectra

The UV absorption spectra of bovine serum albumin in 0.01 M PBS in absence and presence of nicotine are shown in **Figure 2.** With the addition of nicotine, the absorption

Figure 1. The fluorescence for 6.2×10^{-6} mol/L BSA with increase of [nicotine]: 0, 0.4, 0.6, 0.8, 1. 6, 3.2 (×25/8mmol/L), from top to bottom. Excitation wavelength 285 nm.

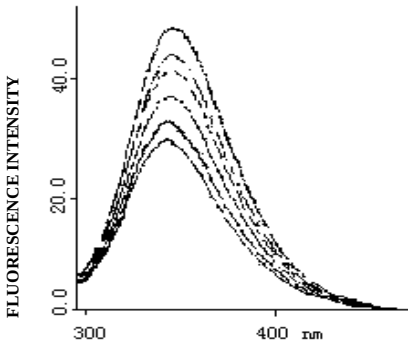
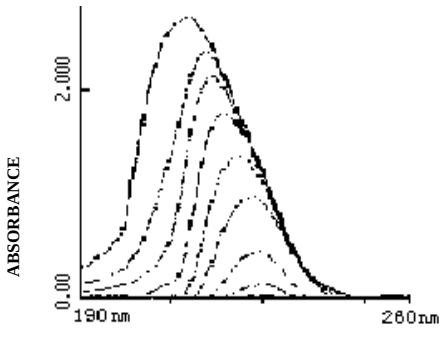


Figure 2. Changes in the UV absorbing spectra of 3×10^{-6} mol/L BSA in 0.01 mol/L PBS with increase of [nicotine]: 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 9.6(×25/8mmol/L), from top to bottom.



band around 210 nm decreased, shifted to longer wavelengths and narrowed. The absorption band of 210 nm represents the absorption of peptide groups of protein. The change of absorption spectrum is owing to the change of the conformation of protein,

which is caused by the binding of BSA with nicotine.

In the case of native bovine albumin, higher molar ratios result in blue shifts in the region of 250-320 nm. The 210 nm spectral changes can be analyzed by the following Hildebrand-Benesi equation based on the 1:1 complex[5]:

$$\frac{1}{\Delta A} = \frac{1}{\alpha \cdot K_a [G_0]} + \frac{1}{\alpha}$$

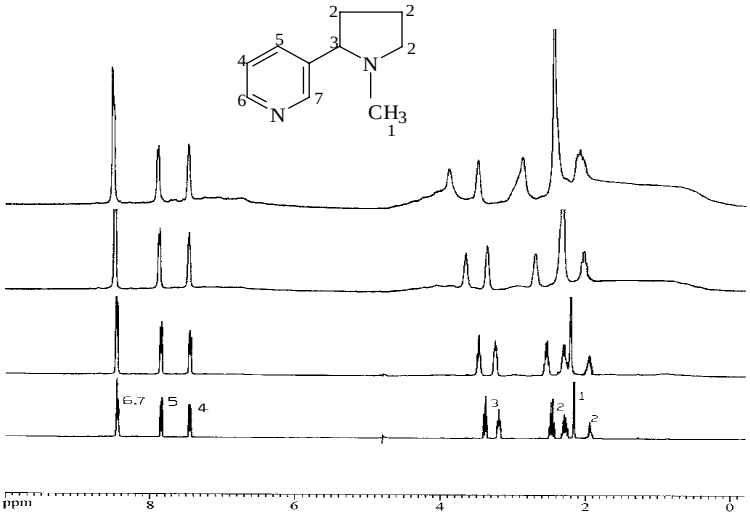
where $\alpha=[H_0] \cdot \varepsilon_0$, K_a is association constant, $[G_0]$ is the concentration of nicotine, ΔA is the change of absorption intensity of BSA by nicotine. There is a good linear relationship between $1/\Delta A$ and $1/[G_0]$, r=0.9942, indicates the association number is one, the association constant is 287.1 l/mol.

BSA contains two tryptophan residues, but nicotine only bound to one tryptophan residue. It can be explained that the conformational change results in transfer of one of the tryptophan residues from a position inside of a hydrophobic part of the protein molecule to an exterior, solvent-exposed part, and in change of the vicinity of the other tryptophan residue in a contrary way.

^{1}H NMR spectra

^{1}H NMR spectra of nicotine in the absence and presence of different quantity of BSA are shown in **Figure 3**. The ^{1}H NMR of nicotine is at the bottom in **Figure 3**, our assignments are made according to those given in standard ^{1}H NMR spectra handbook. As the ratio of

Figure 3. ^{1}H NMR spectra of 8 mg/ml nicotine at 400 MHz resolution with increase of [BSA]: 0, 10, 40, 80 mg/ml, from bottom to top, in 20 mmol/L phosphate-D_2O buffer, at 24°C.



BSA to nicotine gradually increased, all peaks shifted more and more to downfield, and became broader and broader. At high concentration of BSA the spin-spin coupling

splitting turned to be barely discernible. But the most remarkable change of ^{1}H NMR spectra was that of proton 3 of nicotine, the next were proton 2 and 1of nicotine. When the molar ratio of BSA to nicotine is 0.1, the chemical shift change of proton 3 is 0.16ppm, but the chemical shift changes of pyridine protons were unremarkable. This means the interaction of pyrrolidinyl in nicotine with BSA was much stronger than that of pyridine. The downfield shifting of the proton peaks of nicotine by BSA may be explained by the deshielding of the former by the latter. The phenomenon is similar to the groove-binding mode between small molecules and DNA[6]. When nicotine bound to BSA, the tumbling motion of the complex molecules will be slower than that of the free nicotine molecules, resulting in the peak broadening of all proton peaks of nicotine. That is exactly what is shown in **Figure 3.**

In conclusion, the speculated binding of nicotine to a tryptophan residue in albumin might be accounted for the important function of this protein in carcinogen transport or detoxification. Serum albumin in blood could function to protect nicotine against solvolysis and transport it from the liver to extra hepatic target tissues such as the mammary gland and kidney. In view of the abundance of serum albumin in blood and in liver, the binding of nicotine to BSA could represent a significant pathway of carcinogen dotoxification.

Acknowledgments

We thank the financial support of The National Natural Science Foundation of China and Research Fund for Doctoral Program of Higher Education. We thank Professor Yuan Fang LIU and Professor Xiang Yun WANG for their help in this work.

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Received 16 October 1999