INVESTIGATION OF THE INTERACTION BETWEEN NAPROXEN AND HUMAN SERUM ALBUMIN

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Abstract. Human serum albumin (HSA) is the principal extracellular protein with a high concentration in the blood plasma and carrier for many drugs to different molecular targets. This work was designed to examine the interaction of human serum albumin (HSA) with naproxen (NAP) in aqueous solution, using a constant protein concentration of 1.25 μM and various naproxen contents of 4 –200 μM. UV-vis spectroscopy was used to determine the binding mode. The binding constant and the number of binding sites were obtained using an identical independent-binding sites model, which assumes that each HSA molecule has *n* low-affinity binding sites, each with the same binding constant K_d.

Key words: Human serum albumin naproxen, binding mode, UV-vis spectroscopy.

INTRODUCTION

Studies on the binding of drugs to protein are of great importance in biological, biomedical and pharmaceutical sciences. Among investigated proteins, a special attention was paid to human serum albumin (HSA), the principal extracellular protein in the blood plasma. Its main physiological function consists in the storage and transportation of a wide range of endogenous and exogenous compounds. The physiological importance of HSA and the relative ease with which it can be isolated and purified on a large scale, determined the usefulness of HSA as a model protein for the study of ligand-protein interactions. Drug-human serum albumin interaction has been intensively studied because this interaction can serve as a means of drug storage, control of drug delivery to tissue receptors and prevention of the drug from being metabolized rapidly.

Human serum albumin interacts with drugs through its binding sites. There are mainly two classes of binding sites on the HSA molecule [5], one is the high-affinity binding sites and the other is the low-affinity binding sites.

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The high-affinity binding sites, which have the properties of low capacity, have been the main topic of study for a long time by various analytical techniques [2, 3, 7, 9]. The high-affinity binding sites have been further divided into several groups, according to their selectivity to the drugs. Thus, Sudlow [8] identified two distinct binding sites on albumin for acidic drugs based on their abilities to displace fluorescent markers such as 5-(dimethyl amino)-naphthalene-1-sulfonamide and dansylsarcosine. Sudlow's site I and II are commonly known as the warfarin site and diazepam site respectively. Sudlow site II is the principal binding site for drugs. The binding site is a hydrophobic pocket and has a high affinity for small aromatic ligands which may be neutral or negatively charged on periphery. Binding at this site is usually tight and specific with 1:1 stoichiometry. Sudlow site I consists of several adjacent and overlapping regions, and can bind many diverse ligands. Site I is largely responsible for the reputation of albumin to bind a large and diverse variety of ligands with medium affinity (dissociation constant K_d = 10–100 μM). In addition to the specific sites discussed above, human serum albumin is also recognized for its ability to "soak up" ligands in a non specific manner. These low affinity sites have the properties of high capacity. It means that in this type of binding one HSA molecule can bind many drug molecules but the interaction is very weak. It has found that many drug molecules are involved in this type of low-affinity binding interaction, especially when the drug concentration is much higher than that of HSA in the blood plasma (aprox 45mg/ml). In this case the drug is bound first to the high affinity binding sites and than the drug in excess is bound to the low-affinity binding sites. Both mechanisms of binding contribute to the biological effects of drugs.

In this paper, we studied the low-affinity interaction between HSA and NAP using UV-vis spectroscopy.

MATERIALS AND METHODS

Human serum albumin (factor V, fatty acid free) and naproxen were purchased from Sigma Chemie Gmb H, Germany and used without purification. Double distilled, deionized water was used as solvent.

Two stock solutions were prepared in water: one of 1.25 μM HSA and the other of 1.25 μM HSA and 400 μM naproxen. A first set of samples, each containing 1.25 μM HSA and different naproxen concentrations in the range 4–200 μM were then prepared by mixing the stock solutions in appropriate amounts. A second set of samples containing only naproxen in the same range of concentration as those in the first set were also prepared.

Absorbance UV-vis spectra were recorded along the wavelength range of 240–400 nm, in a 10 mm quartz cell by using a JASCO-V550 spectrophotometer, under the following conditions: scan rate 200 nm/min, data pitch 0.2 nm, spectral bandwidth 2 nm. A careful control of the temperature was gained by a cooling device, to keep the temperature at 25ºC.

The HSA-naproxen interaction was analysed using a simple two-site model [6] in which the ligand L (naproxen) can bind to any of the n identical independent binding sites on a protein P (HSA), according to the equilibrium

$$P + L \underset{K_d}{\rightleftharpoons} PL \tag{1}$$

with the dissociation constant:

$$K_d = \frac{[P][L]}{[PL]} \tag{2}$$

The observed absorbance A_{obs}, is given by:

$$A_{obs} = A_L + A_P + A_{PL} \tag{3}$$

Taking into account that the HSA concentration is much smaller than that of naproxen and the experimental data were collected at λ = 330.4 nm, which is far away from the maximum absorbance of HSA (A_{HSA}^{max} = 0.043 at λ = 278.5 nm) we have:

$$A_{obs} = A_L + A_{PL} = \varepsilon_f l[L_f] + \varepsilon_b l[L_b] \tag{4}$$

where $[L_f]$ is the concentration of the free ligand, $[L_b]$ is the concentration of the bound ligand and l is the light path of the cuvette (1cm).

Supposing that each HSA molecule has a minimum of n sites available for accepting ligand molecules, and the total concentrations of HSA and ligand in solution are C_P and C_L, respectively, we have:

$$nC_P = [P] + [L_b] \tag{5}$$

$$C_L = [L_f] + [L_b] \tag{6}$$

where $[P]$ is the concentration of the free binding sites of HSA. By displacing $[L_f]$ in Eq. (4) by (6) we have:

$$A_{obs} = \varepsilon_f l C_L + \Delta\varepsilon l[L_b] \tag{7}$$

where $\Delta\varepsilon = \varepsilon_b - \varepsilon_f$ states for the difference in molar absorption coefficients of NAP/HSA and naproxen.

In the absence of HSA we have:

$$A_{obs}^{(0)} = \varepsilon_f l C_L \tag{8}$$

and eq. (7) can be written as:

$$\Delta A = A_{obs} - A_{obs}^{(0)} = \Delta\varepsilon l[L_b] \tag{9}$$

where ΔA is the difference in absorption of the naproxen aqueous solution in the presence and absence of HSA.

By substituting Eqs (5), (6) and (9) into Eq. (2) and taking into account that $[PL] = [L_b]$, we obtain:

$$K_d = \frac{(nC_P - \frac{\Delta A}{\Delta\varepsilon \cdot l})(\Delta\varepsilon \cdot l \cdot C_L - \Delta A)}{\Delta A} \tag{10}$$

When ΔA data as a function of C_L are available, fitting curve with Eq. (10) will simultaneously find the three parameters, n, $\Delta\varepsilon$ and K_d.

RESULTS

The UV-vis absorption spectrum of naproxen in water is typical of a 2-substituted naphthalene compound, presenting a three band system centred around 220 nm, 240–280nm and 310–330 nm.

The UV-vis absorption spectra of naproxen in aqueous solution were recorded across 10–200 μM concentration range in the peak absorbance range of A = 0.1–1. The experimental data were collected at λ = 330.4 nm, far away from HSA maximum absorbance (λ = 278.5 nm).

The naproxen calibration curve was constructed by fitting the observed absorbance $A^{(0)}$ as a function of naproxen concentration with Eq. (8).

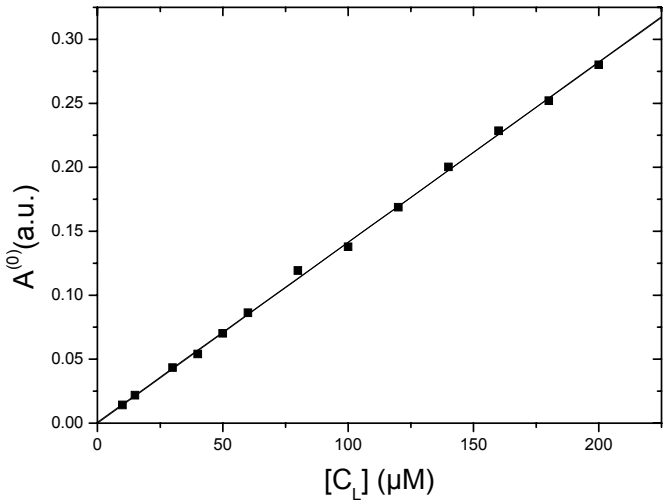

Fig. 1. Plot of naproxen absorbance $A(0)$ *versus* concentration.

As can by seen in Figure 1, the Beer-Lambert law is satisfied over the whole range of naproxen concentrations, the obtained results being: $\varepsilon_{NAP} = 1410\ cm^{-1}M^{-1}$, $r = 0.99958$, where r is the correlation coefficient.

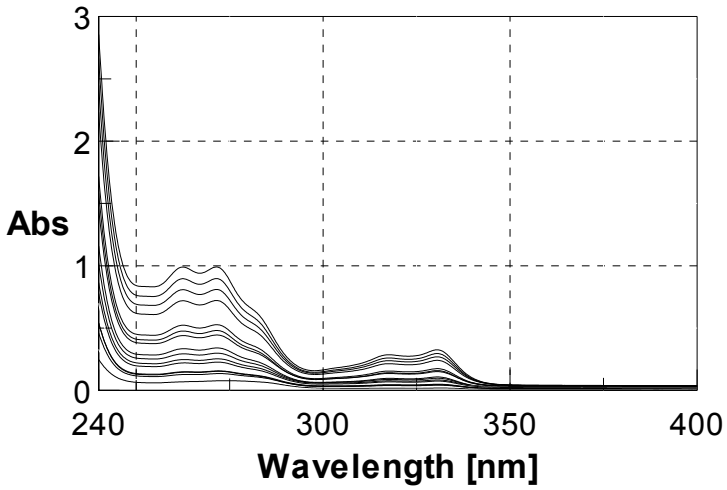

Fig. 2. Absorption spectra of naproxen at increasing concentrations in the presence of 1.25 μM HSA.

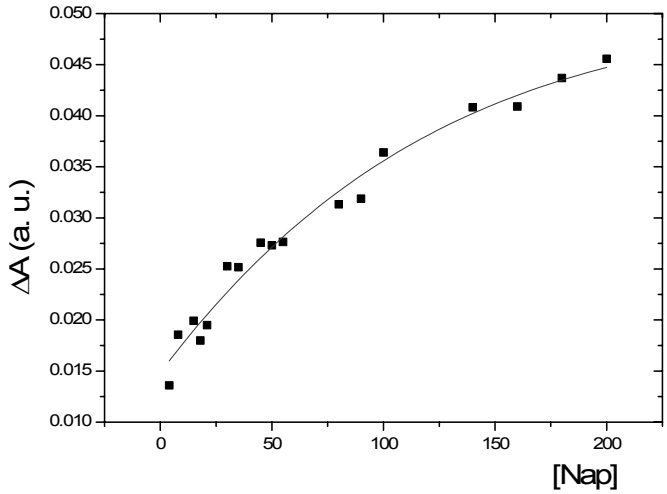

Fig. 3. Plot ΔA as a function of naproxen concentration. The solid curve is the best-fit calculated curve.

The second set of UV-vis measurements were performed on samples containing 1.25 μM HSA and different naproxen concentrations in the range 10–200 μM.

Figure 2 shows the UV-vis spectra for increasing naproxen concentrations in the presence of 1.25 μM HSA.

The experimental data were collected at the same wavelength (λ = 330.4 nm) as for pure naproxen.

In Figure 3, ΔA, the difference in absorption of naproxen aqueous solution, in the presence and absence of human serum albumin, is plotted versus naproxen concentration.

As can seen from Eq (10), developing this expression in terms of ΔA, a quadratic equation is obtained that can by solved and expressed as a function of K_d, $\Delta\varepsilon$ and n as

$$\Delta A = 0.5\Delta\varepsilon\left\{\left(C_L + nC_P + K_d\right) \pm \sqrt{\left(C_L + nC_P + K_d\right)^2 - 4nC_LC_P}\right\} \tag{11}$$

From the two possible solutions, only that obtained using minus sign in front of the square root has physical meaning. The solid line in Figure 3 is the best-fit calculated curve with Eq. (11), the fitting parameters being

$$\begin{cases} K_d = 23.7\mu M \\ n = 5 \\ \Delta\varepsilon = 7394.7 cm^{-1}M^{-1} \end{cases} \tag{12}$$

Taking into account that $\Delta\varepsilon = \varepsilon_b - \varepsilon_f$, the molar absorptivity for the bound naproxen is $\varepsilon_b = 8804.7\ cm^{-1}M^{-1}$.

In general it is difficult to compare K_d and n for a HSA-ligand system from literature survey because there are often multiple reports for the same system that appears to reach different quantitative conclusions. As Fielding *et al*. [1] pointed out, some of this variance is due to the variety of experimental approaches.

In our case we can mention that Kaneo *et al*. [4] have investigated the low-affinity binding of naproxen to BSA, using equilibrium dialysis. They obtained $n = 7$ and $K_d = 480$ μM.

CONCLUSIONS

The low-affinity interaction between human serum albumin and naproxen was studied using UV-vis spectroscopy. The identical independent-binding sites model was applied to evaluate the dissociation constant K_d and the number of binding sites n of the naproxen/HSA complex. The obtained results indicate that this model can explain well the capacity of low-affinity binding of proteins for the ligands.

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