Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 981–984

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Binding interaction of indomethacin with human serum albumin

M. Bogdan*, A. Pirnau, C. Floare, Carmen Bugeac

National Institute for Research and Development of Isotopic and Molecular Technologies, P.O. Box 700, 400293 Cluj-Napoca, Romania

ARTICLE INFO

Article history:
Received 15 January 2008
Received in revised form 2 April 2008
Accepted 3 April 2008
Available online 10 April 2008

Keywords:
Indomethacin
Human serum albumin
Fluorescence quenching
Binding parameters: energy transfer

ABSTRACT

The interaction between indomethacin and human serum albumin (HSA) was investigated by fluorescence quenching technique and UV–vis absorption spectroscopy. The results of fluorescence titration revealed that indomethacin, strongly quench the intrinsic fluorescence of HSA by static quenching and nonradiative energy transfer. The binding site number n and the apparent binding constant K_A, were calculated using linear and nonlinear fit to the experimental data. The distance r between donor (HSA) and acceptor (indomethacin) was obtained according to fluorescence resonance energy transfer (FRET). The study suggests that the donor and the acceptor are bound at different locations but within the quenching distance.

1. Introduction

Human serum albumin (HSA), is the most abundant protein in blood plasma and is able to bind, and thereby transport, various compounds such as fatty acids, bilirubin, tryptophan, steroids and a large number of drugs [1]. Drug–protein interaction has a great significance in pharmacology. It can affect the biological activity and toxicity [2] of drug. The binding parameters are helpful in the study of pharmacokinetics and the design of dosage forms [3].

Indomethacin, a methylated indole derivative is a member of the arylalkanoic acid class of non-steroidal anti-inflammatory drugs (NSAIDs), which includes diclofenac. It is commonly used to reduce fever, pain, stiffness and swelling. It works by inhibiting the production of prostaglandins molecules, known to cause these effects. The drug–protein interactions are often monitored by spectroscopic techniques because these methods are sensitive and relatively easy to use. They have advantages over conventional approaches such as affinity and size exclusion chromatography, equilibrium dialysis and ultrafiltration [4,5], which suffer from lack of sensitivity and relatively long analysis time. Molecular interactions between serum albumins and many NSAIDs have been investigated successfully [6–10], but detailed investigations of the interaction of human serum albumin with indomethacin are scant [11,12].

In this study, we investigated the binding reaction and the effect of energy transfer between HSA and indomethacin by spectrofluorimetry.

2. Materials and methods

2.1. Materials

Human serum albumin (fatty acid free, 99%) fraction V and indomethacin (≥99%) were purchased from Sigma–Aldrich Chemie GmbH. Doubly distilled water was used in all experiments.

2.2. Apparatus

Fluorescence measurements were performed at room temperature on a JASCO-6500 spectrofluorimeter equipped with a 150 W Xenon lamp. The fluorescence emission spectra were recorded in the wavelength of 300–500 nm, upon excitation at 280 nm, and both excitation and emission bandwidth was 3 nm. The absorption spectrum of 10^{-5} M free indomethacin was recorded at room temperature on a double beam JASCO V-550 spectrophotometer from 200 to 500 nm.

2.3. Procedures

The solutions were prepared at room temperature using doubly distilled water. The concentration of HSA was kept fixed at 5×10^{-6} M and the indomethacin concentration was varied from 0 to 3.5×10^{-5} M.

* Corresponding author. Tel.: +40 264 584037; fax: +40 264 420042.
E-mail address: mircea@s3.itim-cj.ro (M. Bogdan).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.04.003

3. Results and discussion

3.1. Fluorescence quenching

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecules. Fluorescence quenching can be caused both by collisions (dynamic) and complexes formation (static) with the quencher [13]. The effect of indomethacin on HSA fluorescence intensity is shown in Fig. 1. It could be seen that the fluorescence intensity of HSA dropped regularly with the increase of indomethacin concentration, along with a slight blue shift from 330 to 320 nm. This observation implies that the fluorescence quenching process may be mainly controlled by a static quenching mechanism rather than a dynamic one.

The fluorescence quenching data are usually analyzed by the Stern–Volmer equation [14]:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence emission peak area of HSA without or with quencher respectively, k_q, K_{SV}, τ_0 and [Q] are the quenching rate constant of a biomolecule, the Stern–Volmer quenching constant, the average lifetime of the biomolecule without quencher (for most biomolecules τ_0 is about 10^{-8} s) and the concentration of free quencher. Because the free indomethacin concentration is not known in our experiment it was approximated by the total concentration of indomethacin. As can be seen in Fig. 2, the plot of F_0/F for HSA versus indomethacin concentration exhibit a good linearity (R = 0.9984) and affords K_{SV} and k_q to be $(5.04 \pm 0.1)10^4\ M^{-1}$ and $(5.04 \pm 0.1)10^{12}\ M^{-1}\ s^{-1}$.

However, the maximum diffusion collision quenching rate constant of various quenchers with the biopolymer is $2.0 \times 10^{10}\ M^{-1}\ s^{-1}$ [15]. Obviously, the derived value for k_q is two orders of magnitude grater than the maximum diffusion collision quenching rate constant. Therefore, it indicates that the fluorescence quenching process of HSA may be mainly governed by a static quenching mechanism rather a dynamic quenching process.

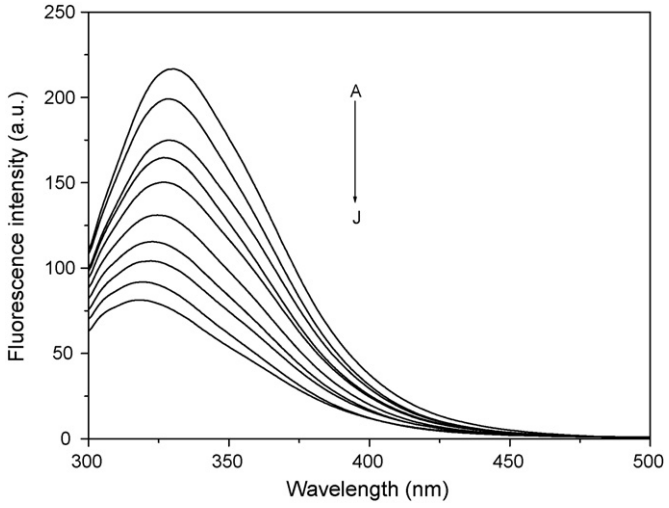

Fig. 1. Emission spectra of HSA in the presence of various indomethacin concentrations. [HSA] = 5.0×10^{-6} M; [Ind]/10^{-6} M, (A–J): 0, 2.5, 5.0, 7.5, 10, 15, 20, 25, 30, 35.

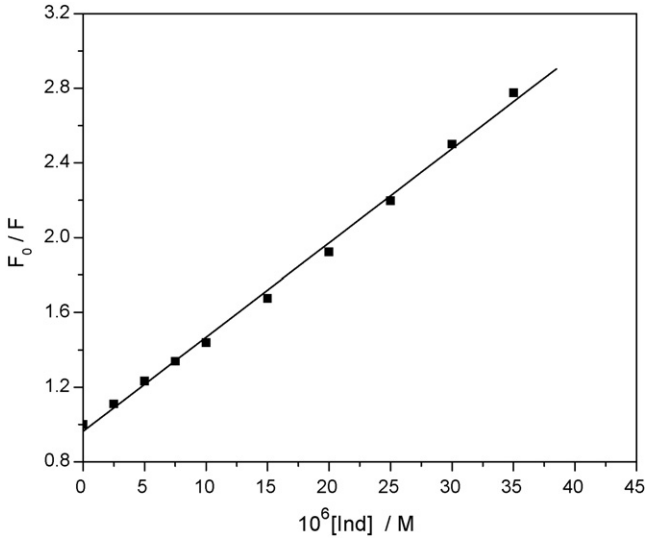

Fig. 2. Stern–Volmer plot for the quenching of HSA fluorescence by indomethacin.

3.2. Apparent association constant and binding sites

For the static quenching process, under the assumption that there are the same and independent binding sites n in the protein, the binding process can be described by

$$P + nD \leftrightarrow D_nP \quad (2)$$

where P is the protein, D is the drug molecule and D_nP is the complex molecule. Because the number of binding site of protein and drug is n and 1, respectively, the equivalent concentration of the complex is $[D_nP]$, the equivalent concentration of drug is $[D]$ and the equivalent concentration of the protein is $n[P]$. In this case the binding constant is given by

$$K = \frac{n[D_nP]}{[D]n[P]} \quad (3)$$

and the following relationship could be made from the stoichiometric coefficient of Eq. (2):

$$[P_t] = [P] + [D_nP] \quad (4)$$

$$[D_t] = [D] + n[D_nP] \quad (5)$$

$[P_t]$ and $[D_t]$ are the total concentration of protein and drug, respectively. If the fluorescence is caused only by the protein, the relationship between the protein concentration and the fluorescence peak area can be described by

$$\frac{F_0}{F} = \frac{[P_t]}{[P]} \quad (6)$$

Therefore, the relationship between the fluorescence peak area and the total drug concentration can be deduced [16] as:

$$\frac{F_0}{F} = K[D_t]\frac{F_0}{F_0 - F} - nK[P_t] \quad (7)$$

It follows that by keeping fixed the total protein concentration and varying the total drug concentration, from the plot of F_0/F as a function of $[D_t]F_0/(F_0 - F)$, the binding constant K and the binding site n can be obtained by using a least-squares algorithm.

For the reaction described by Eq. (2), many equations in expressing the relationship of fluorescence intensity and drug concentration were derived [16–18]. In general, in these equations, the free drug concentration $[D]$ is used to establish the formula, but $[D]$ is not known in experiment. A nonlinear equation was derived by

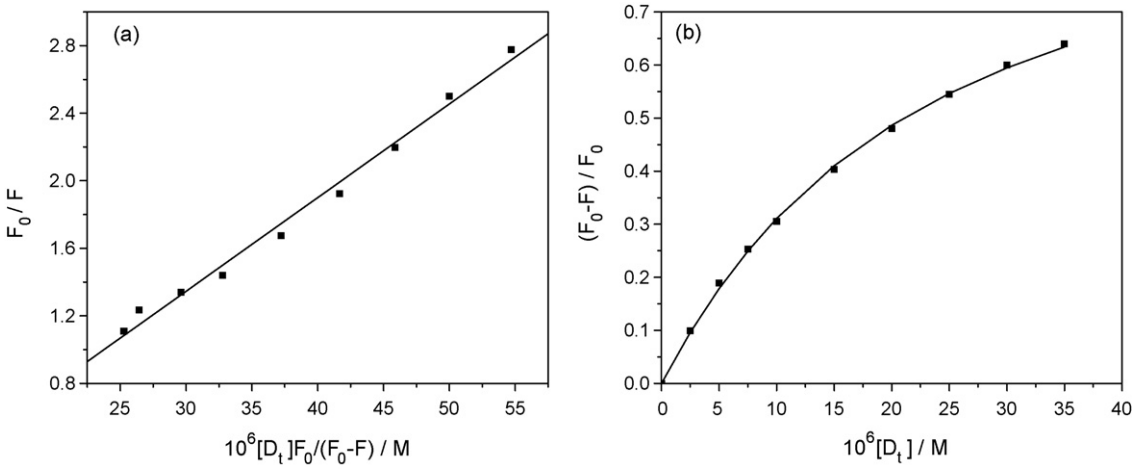

Fig. 3. Fitting curve of HSA–indomethacin system. (a): fitting curve of Eq. (7); (b): fitting curve of Eq. (8).

Guo et al. [18] based on Scatchard's site binding model and fluorescence quenching. This equation is given by:

$$\frac{\Delta F}{F_0} = \frac{(A+B) - \sqrt{(A+B)^2 - 4A}}{2} \tag{8}$$

where

$$A = \frac{[D_t]}{n[P_t]} \tag{9}$$

$$B = 1 + \frac{1}{Kn[P_t]} \tag{10}$$

Using the experimental data of fluorescence quenching on HSA, a least squares and a nonlinear algorithm was assessed by Eqs. (7) and (8). The fitting curves are shown in Fig. 3 and Table 1 gives the obtained results.

3.3. Energy transfer between HSA and indomethacin

HSA has three intrinsic fluorophores: tryptophan, tyrosine and phenylalanine. In general, the intrinsic fluorescence of HSA is almost contributed by tryptophan alone because phenylalanine has a very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is near an amino group or a tryptophan. That is, the change of intrinsic fluorescence intensity of HSA is that of fluorescence intensity of tryptophan residue located in subdomain II A, when small molecules are bound to HSA. The distance between the tryptophan residue and the bound indomethacin could be determined using fluorescence resonance energy transfer (FRET). FRET occurs whenever the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor. The overlap of the UV absorption spectrum of indomethacin with the fluorescence emission spectrum of HSA is shown in Fig. 4.

According to Förster's theory [19], the energy transfer efficiency E, depends not only on the distance r between the donor and the acceptor, but also on the critical energy transfer distance R_0, expressed by the relation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \tag{11}$$

where R_0 is the critical distance, when the transfer efficiency is 50%. R_0, in units of cm^6, is expressed as:

$$R_0^6 = 8.8 \times 10^{-25}[k^2 N^{-4} \Phi_D J(\lambda)] \tag{12}$$

where k^2 is the factor expressing the relative orientation of the donor to the acceptor molecule, N is the refractive index of the medium, Φ_D is the quantum yield of the donor in the absence of the acceptor and $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor in units of $M^{-1}\,cm^3$. The spectral overlap integral $J(\lambda)$ for a donor–acceptor pair is defined as:

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \tag{13}$$

where $F_D(\lambda)$ is the corrected fluorescence intensity of the donor at wavelengths λ to $\lambda + \Delta\lambda$, with the total intensity normalized to unity and $\varepsilon_A(\lambda)$, the molar extinction coefficient of the acceptor at wavelength λ. By integrating the overlap spectra in Fig. 4 for λ = 300–500 nm, J was estimated to be $3.06 \times 10^{-15}\,M^{-1}\,cm^3$.

Table 1
Binding parameters for HSA–indomethacin system

Equation	Binding constant ($10^4\,M^{-1}$)	Binding site	Correlation coefficient
(7)	5.549 ± 0.213	1.152 ± 0.30	0.9949
(8)	5.573 ± 0.240	1.230 ± 0.28	0.9996

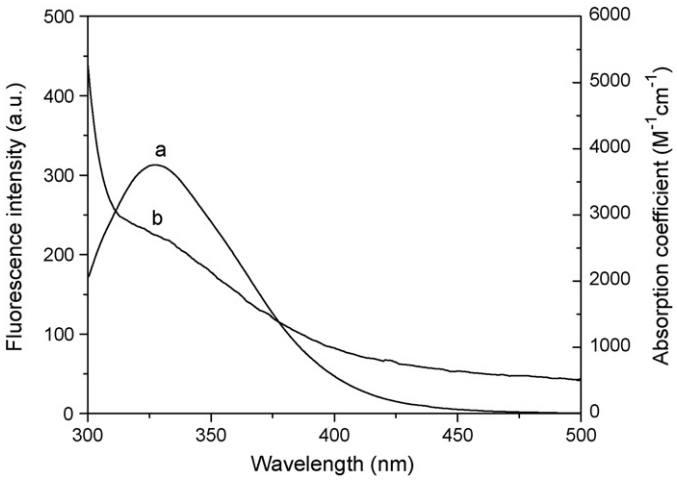

Fig. 4. The overlap of the fluorescence spectrum of HSA (a), and the absorption spectrum of indomethacin (b). [HSA] = [Ind] = 10^{-5} M.

In our case, $k^2 = 2/3$, $N = 1.361$ and $\Phi_D = 0.14$ [20]. Using the above parameters and according to Eqs. (11) and (12) we could calculate that $R_0 = 2.046$ nm; $E = 0.2455$ and $r = 2.47$ nm. The donor–acceptor distance, much shorter than 8 nm [21], indicate that the energy transfer from HSA to indomethacin occurs with high probability.

4. Conclusions

This paper provided an approach for studying the interaction of HSA with indomethacin, using absorption and fluorescence spectroscopy. The results showed that HSA fluorescence quenched by indomethacin through static quenching mechanism. Binding constant of $\sim 10^4\ M^{-1}$ and single binding sites were estimated for this system. The large binding constant value suggests that indomethacin binds to the high affinity binding sites of HSA. Based on the mechanism of Förster energy transfer, the distance $r = 2.47$ nm between HSA and indomethacin was obtained.

References

[1] B. Fichtl, A.V. Nieciecki, K. Walter, Adv. Drug Res. 20 (1991) 117–165.
[2] U. Kragh-Hansen, Pharm. Rev. 33 (1981) 17–53.
[3] A. Rieutord, P. Bourget, G. Troche, J.F. Zazzo, Int. J. Pharm. 119 (1995) 57–64.
[4] D.S. Hage, S.A. Tweed, J. Chromatogr. B 699 (1997) 499–525.
[5] J. Oravcova, B. Böhs, W. Lindner, J. Chromatogr. B 677 (1996) 1–28.
[6] J. Oravcova, V. Mlynarik, S. Bystricky, L. Soltes, P. Szalay, L. Bohacik, T. Trnovec, Chirality 3 (1991) 412–417.
[7] S. Rendik, T. Alebic-Kolbah, F. Kaifez, V. Surijic, Farm. Ed. Sci. 35 (1980) 51–59.
[8] A.M. Evans, R.L. Nation, L.N. Sansom, F. Bochner, A.A. Somogyi, Eur. J. Clin. Pharmacol. 36 (1989) 283–290.
[9] J.-M. Chamouard, J. Barre, S. Urien, G. Houin, J.-P. Tilllement, Biochem. Pharmacol. 34 (1985) 1695–1700.
[10] B. Honore, R. Brodersen, Mol. Pharm. 25 (1984) 137–150.
[11] R.W. Mason, E.G. McQueen, Pharmacology 12 (1974) 12–19.
[12] D. Hultmark, K.O. Borg, R. Elofsson, I. Palmer, Acta Pharm. Suec. 12 (1975) 259–276.
[13] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, second ed., Kluwer Academic/Plenum Publishers, New York, 1999, pp. 238–249.
[14] A. Sharma, S.G. Schulman, Introduction to Fluorescence Spectroscopy, John Wiley & Sons Inc., New York, 1999, pp. 58–59.
[15] W.R. Ware, J. Phys. Chem. 66 (1962) 455–458.
[16] S. Bi, L. Ding, Y. Tian, D. Song, X. Zhou, X. Liu, H. Zhang, J. Mol. Struct. 703 (2004) 37–45.
[17] S. Bi, D. Song, Y. Tian, X. Zhou, Z. Liu, H. Zhang, Spectrochim. Acta A 61 (2005) 629–636.
[18] M. Guo, J.W. Zou, P.G. Yi, Z.C. Shang, G.X. Hu, Q.S. Yu, Anal. Sci. 20 (2005) 465–470.
[19] T. Förster, O. Sinanoglu (Eds.), Modern Quantum Chemistry, Vol. 3, Academic Press, New York, 1996, pp. 93–138.
[20] P. Das, A. Mallick, B. Haldar, A. Chakrabarty, N. Chattopadhyay, J. Chem. Sci. 119 (2007) 77–82.
[21] B. Valeur, J.C. Brochon, New Trends in Fluorescence Spectroscopy, sixth ed., Springer Press, Berlin, 1999, pp. 25–28.