Scientific paper

Enhanced Transport of Nandrolone Decanoate Drug by Human Serum Albumin in Presence of [BMIM]PF₆ and [BMIM]BF₄

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Abstract

The interaction of nandrolone decanoate drug dissolved in (2%) [BMIM]BF₄ or [BMIM]PF₆ with human serum albumin (HSA) at different temperatures in the range of 285–310 K was examined by fluorescence quenching. Stern-Volmer equation and its modified form were used to determine the interaction parameters *K* and *n*. The results revealed that binding affinities of HSA for nandrolone decanoate drug in 2% [BMIM]BF₄ or [BMIM]PF₆ are in the order of 10⁵ M⁻¹ and the number of bound drug molecules per HSA macromolecule are approximated to 1 at all temperatures studied. The thermodynamic parameters: free energy change (ΔG°), enthalpy change (ΔH°) and entropy change (ΔS°) for HSA–nandrolone decanoate/ionic liquid were calculated according to van't Hoff equation. Data analysis indicated that both electrostatic interactions and hydrophobic interactions played important roles in the interaction of nandrolone decanoate drug with HSA.

Keywords: Nandrolone decanoate; Human serum albumin; Ionic liquids; Fluorescence quenching; electrostatic interaction

1. Introduction

The nature and types of interaction between small molecules and biomacromolecules characterize an active area of research. The circulations, free concentration as well as the metabolism of a variety of drugs in human being are powerfully influenced by drug–protein interactions.^{1–5} These interactions can also regulate the drug stability as well as toxicity for the duration of the chemotherapeutic course of action.¹

Ionic liquids are remarkable for their tunable nature and exceptional properties. They have attracted considerable attention in various areas of chemistry, synthesis and catalysis,¹ electrochemistry,^{5–7} biomass conversion,^{8,9} biotechnology^{10,11} and many other fields. The studies on ionic liquids cover a wide range of topics that include structure and properties as well as advanced chemical transformations.^{12,13} Improvements of separation,¹⁴ electrochemical behavior,¹⁵ analytics, extraction, biocatalytic properties¹⁶ are some established areas. The ecological impact of ionic liquids has been recently recognized.^{17,18}

There are three generations of ionic liquids depending on their chemical structure and properties.¹⁹ Limited water solubility and low dissolution rate are among the major problems of modern drug development. The advantages of using ionic liquids in medicinal chemistry has been recently emphasized.^{20–22} Striking solvent abilities of ionic liquids are widely known and are being exploited in extraction^{23–25} and dissolution of biomolecules.^{26–29} Ionic liquids can enhance water solubility of hydrophobic compounds, possibly due to formation of associates between ionic liquids and biomolecules.³⁰

Nandrolone decanoate (Fig. 1) is a parenteral anabolic steroid. It is primarily used to treat anemia, chronic

Figure 1. Chemical structure of nandrolone decanoate

renal failure, osteoporosis and AIDS-associated wasting syndrome. It is freely soluble in ethanol, chloroform and acetone but practically insoluble in water (3.09 mg/mL).

Considering limited solubility of the drug in water, it is of interest to study the effect of some ionic liquids, namely 1-butyl-3-methyl-1-imidazolium tetrafluoroborate [BMIM][BF₄] (Fig. 2.a) and 1-butyl-3-methyl-1-imidazolium hexafluorophosphate [BMIM][PF₆] (Fig. 2.b) to increase its solubility in water and to enhance its ability to transfer the drug through the body. Their behaviors were examined by following and analyzing the fluorescence spectra of human serum albumin-nandrolone decanoate mixture in the absence and presence of the selected ionic liquids as solvents. Since the changes in the fluorescence of HSA are indeed related to changes in the fluorescence of tryptophan residue caused by interactions with a variety of quenchers.

In this work, the quenching of HSA fluorescence intensities by the studied drug/2% ionic liquid systems was explored for the first time at different temperatures in the range of 285–310 K.

Binding parameters (K and n) were determined and thermodynamic parameters of HSA– nandrolone decanoate /ionic liquid interaction were calculated. Data analysis indicated that electrostatic interactions and hydrophobic associations played different roles for the two ionic liquids.

Figure 2. Molecular structure of two ionic liquids: (a) 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF₄); (b) 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM]PF₆)

2. Experimental

2.1. Materials and Solutions

Nandrolone decanoate as an ampoule dissolved in oily solution was obtained from Birzeit Pharmaceutical Company (BPC). The ionic liquids; 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF₄) and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM]PF₆) were obtained from Aldrich (mass fraction > 99%). Human serum albumin (HSA, fatty acid free < 0.05%) was purchased from Sigma Chemical Co. and used without further purification. All other reagents of chemically pure grade were commercially available. Doubly distilled water was used throughout. All stock solutions were kept in the dark at 277 K.

Solutions of HSA containing amounts up to 6.5 micromolar were obtained by dilution with buffer containing the ionic liquid and used further with the drug to study its

effect. All HSA solutions were prepared in 50 mM potassium phosphate buffer solutions of pH 7.0 containing 25 mM NaCl to maintain constant ionic strength. Considering large molar absorptivity of HSA, the solutions were properly diluted to give absorbance in the range 0.2 to 0.7 in the linear response covered by the instrument. The HSA stock solution was kept in the dark at 281 K and the pH was checked with a suitably standardized pH meter. During the protein-drug binding experiments, the HSA concentrations were fixed at 2 μM and the drug concentrations were varied from 0 up to 6.5 μM.

2.2. Apparatus

Lambda 20, a Perkin Elmer UV/Vis spectrophotometer, was used for determination of the concentration of HSA using $\epsilon = 35219 \text{ M}^{-1}\text{cm}^{-1}$ at 278 nm as the molar extinction coefficient of HSA.³¹ Fluorescence spectra were obtained using Perkin Elmer luminescence (series no. 70412) spectrometer equipped with a water-jacketed cuvette holder that was maintained at each chosen temperature by means of a circulatory water bath.

2.3. Fluorescence Measurements

Fluorescence spectra (in the range of 280–450 nm upon excitation at 280 nm) were recorded at various temperatures; 285, 293, 303 and 310 K. The reactants were kept at the same temperature before mixing and making fluorescence measurements. The fluorescence intensities were automatically recorded by injecting 20 μL samples of 80 μM drug solution containing 2% ionic liquids into the HSA solution which makes the final concentration of the test solution 0.148% considering the dilution effect. Each titration was made three times.

The inner-filter effect corrections³² were done according to the formula:

$$F_{\text{corr}} = F_{\text{obs}} 10^{\frac{A_{\text{exc}} + A_{\text{em}}}{2}} \quad (1)$$

where F_{corr} is the corrected fluorescence value, F_{obs} the measured fluorescence value, A_{exc} and A_{em} the measured absorbance values at the excitation and emission wavelengths, respectively. The interval between every two consecutive sample additions was 2 minutes.

3. Results and Discussion

Fluorescence data were corrected for the inner filter effect caused by attenuation of the excitation beam and emission signal because of absorption by quencher and fluorophore to obtain the proper fluorescence intensity values. The value of the correction factor was 1.05 that incurs a negligible effect to the results.

Nandrolone decanoate drug was selected as a model drug due to its limited solubility in water. In addition, organic solvent may cause denaturation to HSA. Therefore, utilization of selected ionic liquids is justified.

This study is on the effect of ionic liquids on drug-protein interaction. The interaction between nandrolone decanoate and HSA in absence of ionic liquids showed very little quenching in fluorescence intensity which is an indication of marginal interaction. On addition of ionic liquid to the nandrolone decanoate-HSA mixture, a notable drop in fluorescence intensity indicating remarkable effect of ionic liquid on the drug-protein interaction.

3. 1. Interaction of Nandrolone Decanoate Drug with HSA in Presence of Ionic Liquids

Emission spectra of the protein's intrinsic fluorescence was performed to investigate the interaction between the drug (nandrolone decanoate) in 2% ionic liquid ([BMIM]BF₄ or [BMIM]PF₆) and HSA at four different temperatures: 285, 293, 303 and 310 K.

The fluorescence spectra of HSA (2 μ M), obtained in the emission range of 250–450 nm in the absence and the presence of increasing nandrolone decanoate concentrations (0–6.5 μ M) containing 2% ionic liquids ([BMIM]BF₄ or [BMIM]PF₆) were studied in phosphate buffer at pH 7.0 and are shown in Figs. 3 and 4, respectively.

The fluorescence spectrum of HSA produced an emission maximum at 343 nm due to the presence of sole tryptophan (Trp-214) residue which is located in subdomain IIA.³³

Addition of nandrolone decanoate to HSA resulted in a gradual decrease in the fluorescence intensity. However, no shift in the emission maximum was noticed upon nandrolone decanoate addition to HSA. Such quenching in the fluorescence intensity indicated the binding of nandrolone decanoate to HSA, and suggested that the polarity changes in the microenvironment around Trp residue might be responsible for the observed quenching of the fluorescence intensity observed.^{34–37}

Fluorescence quenching of the single tryptophan residue in HSA was used to measure drug-binding affinity. Tryptophan fluorescence is the most frequently examined

Figure 3. Fluorescence quenching spectra of human serum albumin at various concentrations of nandrolone dissolved in 2 % [BMIM]BF₄ ionic Liquid, [HSA] = 2 μ M, [nandrolone]/ionic liquid were: 0.00, 0.58, 1.15, 1.72, 2.28, 2.83, 3.37, 3.91, 4.44, 4.96, 5.48, 5.99 and 6.50 μ M all in phosphate buffer solution 50 mM, 25 mM NaCl, pH = 7.0 at different temperatures: (a) 285K (b) 293 K (c) 303 K and (d) 310 K, λ_{ex} = 280 nm.

among the three intrinsic aromatic fluorophores in HSA molecules to obtain information about conformational changes.

A very small shift (about 3 nm in the wave length of the fluorescence intensity) was observed on addition of increasing amounts of the ionic liquids to the test solution. It is inferred that the protein retains its native three-dimensional structure under the conditions studied in this ex-

periment. In this system, quenching in fluorescence was due to interaction of nandrolone decanoate and HSA but effects of ionic liquids were negligible and the main effect of which was a solubilizing role.

The quenching data were analyzed using the Stern-Volmer equation,

$$F_0/F = 1 + K_{SV} [Q], \quad (2)$$

Figure 4. Fluorescence quenching spectra of human serum albumin at various concentrations of nandrolone dissolved in 2 % [BMIM]PF₆ ionic Liquid, [HSA] = 2 μM, [nandrolone] were: 0.00, 0.58, 1.15, 1.72, 2.28, 2.83, 3.37, 3.91, 4.44, 4.96, 5.48, 5.99 and 6.50 μM all in phosphate buffer solution 50 mM, 25 mM NaCl, pH = 7.0 at different temperatures: (a) 285K (b) 293 K (c) 303 K and (d) 310 K, λ_{ex} = 280 nm.

Table 1: Results of the binding in the nandrolone decanoate/ionic liquid/HSA systems..

Ionic Liquid	Temperature	$K_{SV} \times 10^5$ (K)	$K_a \times 10^5$ (L mol ⁻¹)	R	Binding sites (n)
[BMIM]BF ₄	285.15	3.50	6.73	0.99162	1.36
	293.15	2.73	5.90	0.99385	1.23
	303.15	1.90	3.72	0.99174	1.16
	310.15	1.30	2.63	0.99961	1.17
[BMIM]PF ₆	285.15	1.75	7.00	0.99992	1.35
	293.15	1.51	6.51	0.99996	1.25
	303.15	1.26	5.44	0.99994	1.28
	310.15	1.08	3.80	0.99986	1.11

and its modified form,

$$\log [(F_0 - F)/F] = \log K + n \log [Q] \quad (3)$$

Where F_0 and F are the relative fluorescence intensities in the absence and presence of quencher drug, $[Q]$ is the concentration of quencher, K_{SV} is the Stern-Volmer quenching constant and K is the binding constant for the accessible fluorophores.³²

3. 2. Stern-Volmer Analysis of HSA Interaction with Nandrolone Decanoate Drug

The fluorescence quenching data for nandrolone decanoate –HSA in presence of the two selected ionic liquids were analyzed by Stern–Volmer equation (equation 2).

The values of K_{SV} constants at different temperatures are listed in Table 1. The patterns of the F_0/F versus $[Q]$ (Stern–Volmer) plots for HSA were shown in Figs. 5 and 6. These plots present good linear relationships between F_0/F versus drug concentration.

The fluorescence intensities were quenched by the interaction of HSA with nandrolone decanoate in the presence of the ionic liquids ([BMIM]BF₄ and [BMIM]PF₆).

This behavior is attributed to nandrolone decanoate binding to HAS, which may be facilitated by both ionic liquids due to their hydrophobicity and enhanced transport of the drug to the binding site.

From table 1, it is noticed that the binding constants for the two systems: nandrolone decanoate -HSA in presence of the studied ionic liquids, [BMIM]BF₄ and [BMIM]PF₆, were of the same order of magnitude, being slightly higher with [BMIM]PF₆ as compared to that with the former. This result is in accordance with expectations based on the similarity in structure of the two compounds. The hydrophobicity of hexafluorophosphate is more than that of tetrafluoroborate anion. Therefore, the

Figure 5. The Stern –Volmer plots for the binding of nandrolone drug with in [BMIM]BF₄ at 285 K (□), 293 K (○), 303 K (■) and 313 K (●). λ_{ex} = 280 nm; λ_{em} = 343 nm and [HSA] = 2 μ M.

Figure 6. The Stern–Volmer plots for the binding of nandrolone decanoate drug with HSA in presence of [BMIM]PF₆ at 285 K (□), 293 K (○), 303 K (■) and 313 K (●). λ_{ex} = 280 nm; λ_{em} = 343 nm and [HSA] = 2 μ M.

ionic liquid containing hexafluorophosphate can penetrate into the hydrophobic region of HSA making stronger bonding with higher binding constants and explains the observation that this ionic liquid is more effective on the protein.

3. 3. Modified Stern-Volmer Analysis of Binding Equilibria

Quenching data were also analyzed according to the modified Stern-Volmer equation, equation 3. It is shown in Figs. 7 and 8 that the dependence of $\log [(F_0 - F)/F]$ versus $\log[\text{quencher}]$ is linear with a slope equal to the value of binding sites (n) and y-intercept corresponding to the $\log$ of the effective quenching constant (K). The corresponding results of the linear regression equations at four different temperatures are shown in Table 1. The decrease-

Figure 7. Modified Stern-Volmer plots of $\log [(F_0 - F)/F]$ versus $\log[\text{drug}]$ for nandrolone decanoate -ionic liquid-HSA system at different temperatures; the presence of [BMIM]BF₄ or [BMIM]PF₆, [HSA] = 2 μ M.

Figure 8. Modified Stern-Volmer plots of $\log [(F_0-F)/F]$ versus $\log [\text{drug}]$ for nandrolone-ionic liquid-HSA system at different temperatures; the presence of [BMIM]BF₄ or [BMIM]PF₆, [HSA] = 2 μM .

ing trend of K with increasing temperature is in accordance with the K_{SV} dependence on temperature resulting in a reduction of the stability of the nandrolone decanoate–HSA complex at higher temperatures.

3. 4. Types of interaction force between HSA and the nandrolone drug

The changes of free energy, enthalpy and entropy were estimated using the Van't Hoff's equation:

$$\ln K_a = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (4)$$

and the relationship,

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (5)$$

Using van't Hoff equation that can be rewritten as:

$$\left(\frac{\partial \ln K_{eq}}{\partial (1/T)} \right)_P = -\frac{\Delta H^\circ}{R} \quad (6)$$

Integration produces the equation:

$$\ln \left(\frac{K_2}{K_1} \right) = -\frac{\Delta H^\circ}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad (7)$$

Since:

$$\Delta G^\circ = -RT \ln K_{eq} \quad (8)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

Equating the right sides of the equations produces:

$$\ln K_{eq} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (9)$$

This equation is used for calculation of the equilibrium values obtained for the interaction of drug and HSA.

According to the effective binding constants of nandrolone decanoate drug to HSA obtained at the studied temperatures, the thermodynamic parameters were determined from linear Van't Hoff plot (Fig. 9) and the values are presented in Table 2.

Figure 9. Van't Hoff plot for the interaction of HSA and nandrolone decanoate drug in the presence of ionic liquids; [BMIM]BF₄ (■) (and [BMIM]PF₆ (●))

Table 2: Thermodynamic parameters of the nandrolone decanoate/2% ionic liquid–HSA interaction at different temperatures and pH 7.0.

Ionic Liquid	T, K	$K \times 10^5$ $\text{L} \cdot \text{mol}^{-1}$	ΔG° kJ mol^{-1}	ΔH° kJ mol^{-1}	ΔS° $\text{J mol}^{-1} \text{K}^{-1}$	R
[BMIM]BF ₄	285.15	6.73	–32.55	–21.67	38.16	0.99986
	293.15	5.90	–32.86			
	303.15	3.72	–33.24			
	310.15	2.63	–33.50			
[BMIM]PF ₆	285.15	7.00	–31.90	–27.14	16.70	0.99991
	293.15	6.51	–32.04			
	303.15	5.44	–32.20			
	310.15	3.80	–32.32			

The formation of drug – HSA complexes in presence of the selected ionic liquids were carried out spontaneously through exothermic reaction accompanied by positive ΔS° values. Ross and Subramanian³⁸ have characterized the sign and magnitude of the thermodynamic parameter associated with various kinds of interaction that may take place in protein association processes.

Ionic liquid facilitated binding process of nandrolone decanoate to HSA in aqueous solution is governed by hydrophobic as well as specific interactions (electrostatic and/or hydrogen bond). Binding was characterized by positive entropy and negative enthalpy changes and is more effective in the presence of hexafluorophosphate anions although the size of the anionic part in the [BMIM]BF₄ is smaller and can more easily approaches the hydrophobic core of HSA.

4. Conclusion

The interaction between nandrolone decanoate drug and HSA in presence of ionic liquids [BMIM]BF₄ and [BMIM]PF₆ was investigated through fluorescence quenching methods.

The results indicate that the studied ionic liquids affect the binding of nandrolone decanoate drug to HSA at various temperatures in the range of 285–310 K. It was observed that the binding constants between nandrolone and HSA were decreased with temperature.

In addition, the binding constants in presence of ionic liquid [BMIM]PF₆ is slightly greater than that in presence of [BMIM]BF₄ in the drug solution.

The study opened the door to new avenues in the screening and design of appropriate safe drugs containing ionic liquids that will likely find important applications in modern medical research.

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Povzetek

Raziskovali smo interakcijo zdravila nandrolon dekanat, raztopljenega v 2 % [BMIM]BF₄ ali [BMIM]PF₆, s človeškim serumskim albuminom (HSA) pri različnih temperaturah v območju 285–310 K. Za določitev interakcijskih parametrov K in n smo uporabili Stern-Volmerjevo enačbo in njeno modificirano obliko. Rezultati so pokazali, da je vezavna afiniteta HSA za nandrolon dekanat v 2 % [BMIM]BF₄ ali [BMIM]PF₆ reda velikosti 10⁵ M⁻¹ in število vezanih molekul zdravila na vezavno mesto na makromolekuli HSA približno 1 pri vseh preiskovanih temperaturah.

Iz van't Hoffove enačbe smo izračunali sledeče termodinamske parametre: spremembo standardne Gibsove proste entalpije (ΔG°), entalpije (ΔH°) in entropije (ΔS°) za sistem HSA– nandrolon dekanat/ionska tekočina. Analiza podatkov je pokazala, da tako elektrostatske kot hidrofobne interakcije igrajo pomembno vlogo v interakciji nandrolon dekanata s HSA.