Journal of Molecular Structure 995 (2011) 35–40

Contents lists available at ScienceDirect

Journal of Molecular Structure

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

Effects of glycation on meloxicam binding to human serum albumin

Lilianna Trynda-Lemiesz, Katarzyna Wiglusz *

Department of Analytical Chemistry, Faculty of Pharmacy, Wroclaw Medical University, Szewska 38, 50-139 Wroclaw, Poland

ARTICLE INFO

Article history:
Received 9 December 2010
Received in revised form 14 March 2011
Accepted 14 March 2011
Available online 22 March 2011

Keywords:
Meloxicam
Human serum albumin
Glycated albumin
Circular dichroism
Fluorescence

ABSTRACT

The current study reports a binding of meloxicam a pharmacologically important new generation, non-steroidal anti-inflammatory drug to glycated form of the human serum albumin (HSA). The interaction of the meloxicam with nonglycated and glycated albumin has been studied at pH 7.4 in 0.05 M sodium phosphate buffer with 0.1 M NaCl, using fluorescence quenching technique and circular dichroism spectroscopy.

Results of the present study have shown that the meloxicam could bind both forms of albumin glycated and nonglycated at a site, which was close to the tryptophan residues. Similarly, how for native albumin glycated form has had one high affinity site for the drug with association constants of the order of $10^5\ M^{-1}$. The glycation process of the HSA significantly has affected the impact of the meloxicam on the binding of other ligands such as warfarin and bilirubin. The affinity of the glycated albumin for bilirubin as for native albumin has been reduced by meloxicam but observed effect was weaker by half (about 20%) compared with nonglycated albumin. In contrast to the native albumin meloxicam binding to glycated form of the protein only slightly affected the binding of warfarin. It seemed possible that the effects on warfarin binding might be entirely attributable to the Lys 199 modification which was in site I.

1. Introduction

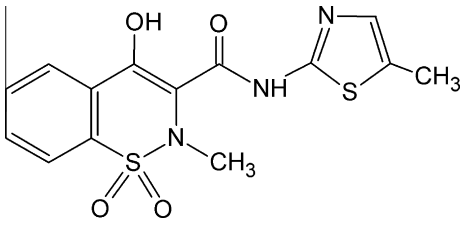
The most drugs are transported in the circulatory system as a complex with an albumin [1]. This is an important part of the drug metabolism since the bound fraction of a drug has no pharmacological effect [2]. Reversible attachment to serum proteins significantly modulates the pharmacokinetics (volume of distribution, clearance and elimination half-life) and pharmaco-dynamics (biological activity and toxicology) of many drugs.

The human serum albumin (HSA), a single-chain protein, is composed of three homologous, predominantly helical domains (I–III); each of these is comprised of two subdomains A and B. The A and B subdomains have six and four α-helices, respectively, connected by flexible loops. Its amino acid sequence contains a total of 17 disulfide bridges, one free thiol (Cys 34) and a single tryptophan (Trp 214). The protein binds a wide variety of endogenous ligands, which represent a spectrum of chemically different molecules, including fatty acids, heme, bilirubin and amino acids [3,4]. The albumin acting also as a buffer on the free drugs concentration.

The principal regions of ligand binding sites of the albumin are located in hydrophobic cavities in subdomains IIA and IIIA, which exhibit similar chemistry. According to the Sudlow's nomenclature [5], bulky heterocyclic anions bind to site I (located in subdomain IIA), whereas site II (located in subdomain IIIA) is preferred by aromatic carboxylates with an extended conformation. The IIIA subdomain is the most active in accommodating of many ligands, as for example, digitoxin, ibuprofen and tryptophan, while a warfarin occupies a single site in IIA [6]. The warfarin shares this binding site with a range of other drugs (including phenylbutazone, tolbutamide and indomethacin). Usually, the drugs bind to high-affinity sites with typical association constants in the range of 10^4–$10^6\ M^{-1}$. Bilirubin, a toxic metabolite of heme binds to the albumin with high affinity at a size located at or near loop 4 in the subdomain IIA [7]. Thus, the binding of particular drug molecule to a serum albumin may change considerably binding abilities of the HSA towards other molecules. On the other hand the impact of pathological factors can significantly alter the binding of the drugs. Pathological conditions can be connected with excess of glucose. It is known during diabetes that the HSA and other proteins in (e.g., hemoglobin) can have modifications to their structure due to the addition of glucose [8]. In the circulation, the HSA becomes nonenzymatically glycated by reducing sugars, and the reference range in normal humans is 6–10% glycoalbumin. However, this proportion increases to ~30% in hyperglycemic patients [9]. The glycation is a non-enzymatic reaction that involves the reaction of glucose with free amine groups on the HSA to form a reversible Schiff base linkage, giving a glycosylamine residue which can later undergo an Amadori rearrangement to form a stable fructosamine residue. Further rearrangement and cleavage of this fructosamine can lead to other modifications, known as advanced glycation end products (AGEs). These modifications are of interest because

* Corresponding author.
E-mail address: wiglusz@cheman.am.wroc.pl (K. Wiglusz).

0022-2860/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.molstruc.2011.03.037



Scheme 1. The chemical structure of meloxicam.

the glycation of the HSA can lead to alterations in the binding of this protein to the drugs such as salicylate, ibuprofen, phenytoin, phenylbutazone and warfarin [10,11].

The principal site of a glycation of the albumin is Lys-525, but the lysine residues in positions 199, 281 and 439 are also susceptible to the glycation and the effect of the glycation on structure, function and metabolism of the HSA is primarily caused by blockage of positively charged these amino acid residues. Because, the folded structure of proteins affects their function, conformational changes associated with the glycation of the albumin may alter its normal functions and drugs binding. Determination of the impact of various factors on the interaction of the drugs with the albumin is especially important if the drug is tightly bound (over 90%) to protein.

Meloxicam [4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide] (C14H13N3O4S2) (Scheme 1) is pharmacologically important new generation, non-steroidal anti-inflammatory drug (NSAID) of enolic acid class compounds with minimum adverse gastrointestinal and renal side effects associated with traditional NSAID [12]. The inhibition of the prostaglandin synthesis through the blockade of cyclooxygenase (COX) has been widely accepted as the mechanism of action of these compounds, the so-called coxibs. The meloxicam makes selective inhibition to COX-2 more than COX-1 [13,14]. The primary function of these drugs is anti-inflammatory effect but they can also be used as agents in cancer treatment, because in various types of cancer, COX-2 is over expressed [15]. COX-derived prostaglandins (PGs) have been shown to modulate cell proliferation, apoptosis, angiogenesis, and immune surveillance [16,17]. It has been reported that COX-2 is overexpressed in human tumors of various organs such as colon, breast, prostate, esophagus, pancreas, liver, head, neck, and lungs [18,19].

The plasma protein binding of meloxicam is more than 99% [20]. Meloxicam exhibits a high degree of binding to albumin, a low apparent volume of distribution and a long plasma half-life (>20 h) [21,22]. The nature of interaction of meloxicam with human serum albumin involves strong drug–protein interactions with only one high affinity site located in subdomain IIA, with association constant of the order of $10^5\,M^{-1}$. The binding of meloxicam to serum albumin involves predominantly hydrophobic interactions although hydrogen bonds cannot be excluded [23,24]. Our earlier studies [25] have shown that meloxicam causes destabilization of the warfarin binding site located in subdomain IIA and marked reductions in the affinity of albumin for bilirubin.

In this work, the effect of glycation on meloxicam binding to human serum albumin and the impact of meloxicam on the binding of other ligands was studied by CD and fluorescence spectroscopy methods.

2. Materials and methods

2.1. Materials

High purity HSA >98% (GE) [No. 05420] and bilirubin ⩾95% [No. 14370] were obtained from Fluka BioChemica. Albumin glycated human (2.2 mol hexose/mol protein, gHSA) was obtained from Sigma [No. A8301].

[4-Hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide] (Meloxicam, [No. M3935]), 3-(-acetonylbenzyl)-4-hydroxycoumarin (Warfarin ⩾98%, [No. A2250]) were supplied by Sigma.

The stock solution of meloxicam was prepared by dissolving in minimal amounts of methanol; diluted with doubly distilled water and used in all experiments from freshly prepared 0.2 mM solutions (the maximum methanol content did not exceed 10% v/v). In the final step a stock solution was diluted with the buffer and added drop wise to the protein solution. HSA concentration was determined by absorption spectrum, taking the absorbance of a 1 mg/cm^3 solution at 280 nm as 0.55 [26]. All procedures involving bilirubin were carried out under minimal light. The appropriate amount of the substance was dissolved in 0.1 M NaOH, rapidly diluted tenfold and used in this form within 1 h. Concentrations were determined spectrophotometrically using an absorption coefficient of 52 $mM^{-1}\,cm^{-1}$ at 437 nm [26]. In all of the experiments, a sodium phosphate buffer (0.05 M, pH 7.4) containing 0.1 M NaCl was used.

2.2. CD measurement

Circular dichroism measurements were recorded at 25 °C on a Jasco J-715 spectropolarimeter, over the range of 190–250 and 300–600 nm, using 0.1 and 1.0 cm cuvettes respectively at room temperature. The spectra are expressed as MRE (mean residue ellipticity) in deg cm^2 dmol^{-1} and Mol CD in $dm^{-3}\,dmol^{-1}\,cm^{-1}$ respectively.

2.3. Fluorescence measurement

Emission fluorescence spectra were recorded using SLM Aminco SPF-500 spectro-fluorimeter and 1 cm × 1 cm × 4 cm quartz cells. Tryptophan fluorescence of albumin was measured by exciting the protein solution at 280 (298) nm. The emission spectra were recorded from 300 to 500 nm. Warfarin was measured by exciting at 335 nm and emission wavelength at 378 nm. Blank values corresponding to the buffer were subtracted to correct for the background fluorescence.

2.3.1. Calculation of the binding constant

The binding constant has been calculated by the Trp-214 fluorescence quenching of unmodified (native) and glycated HSA. The binding experiments were performed under identical conditions for the two albumin forms.

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore, which is induced by a variety of molecular interactions with a quencher molecule. Fluorescence quenching can be dynamic, resulting from collisional encounters between the fluorophore and the quencher, or static, resulting from the formation of a ground state complex between the fluorophore and the quencher [27].

Fluorescence quenching can be described by the Stern–Volmer equation:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q]$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively, k_q is the bimolecular quenching constant, τ_0 is the lifetime of the fluorophore in the absence of the quencher and the fluorescence lifetime of the biopolymer is 10^{-8} s [27], [Q] is the concentration of the quencher, and K_{SV} is the Stern–Volmer quenching constant. Hence, this dependence was applied to determine K_{SV} by linear regression of a plot of F_0/F

against [Q]. As to the fluorescence of meloxicam, no fluorescence was observed in this excitation range.

3. Results and discussion

3.1. Structural properties

The effect of the glycation on structure, function and metabolism of the HSA is primarily caused by blockage of positively charged amino acid residues, e.g. removal of the positive charges of lysine in positions 199, 439 and 525. It is known that glycation had effect on the overall conformation of the HSA. The structural properties of glycated HSA (gHSA) were examined by UV CD spectra (Fig. 1). The albumin structure is predominantly α-helical. Approximately, 67% of the HSA is helical, the number of helices in the structure is 28 [4]. The remaining secondary structure consists of 10% b-turns and 23% extended peptide chain.

The CD spectra of the HSA exhibited two negative bands in the ultraviolet region at 209 and 222 nm characteristic for a α-helical structure of the protein. Fig. 1A indicates that modification only imperceptibly decreases both of these bands. The magnitude of the CD change can suggest that glycation does not alter considerably the overall α-helical structure of HSA (about 5–6% compared with native protein). These results are consistent with earlier structural studies of this protein [28]. In contrast to CD results (examination of secondary structure) conformational change in the environment of Trp-214 appears to be significant (Fig. 1B). HSA contain only one tryptophan residue (Trp-214), which resides in subdomain IIA. Changes in emission spectra of tryptophan are common in response to protein conformational transitions. The relative fluorescence intensities for the glycated protein are reduced to about 60%. The changes of tryptophan fluorescence observed for the glycated protein could result from various causes such as changed location of the tryptophan ring, possible interaction with other amino acid residue or overall protein conformation changes.

3.2. Analysis of fluorescence quenching of glycated HSA by meloxicam

Changes in emission spectra of tryptophan in HSA molecule are common in response to protein conformational transitions. Ligands that bind to albumin particularly to the region containing this Trp residue cause a fluorescence quenching of albumin when excited at 298 nm. When 280 nm excitation wavelength is used, fluorescence of albumin comes from both tryptophanyl and tyrosyl residues. Thus, meloxicam can bind to albumin at a site, which is close to the tryptophan residues [23,25].

The comparable changes in the fluorescence emission spectra at 340 nm on excitation at 280 and 298 nm, indicating that tyrosine residues are not involved in the binding [25].

The binding of meloxicam with native (Fig. 2B) and glycated HSA (Fig. 2C) at pH 7.4 causes a concentration-dependant decrease in the protein intrinsic fluorescence. It could be seen that the fluorescence intensity of HSA dropped regularly with the increase in meloxicam concentration, indicating that meloxicam can bind to the HSA at the native and modified state and quench the Trp intrinsic fluorescence intensity. If the ligand can quench the tryptophan residues, the residues must be located in or near the binding position. Fig. 2 shows that the maximum fluorescence intensity decreases near 335 nm, so the binding position between meloxicam and serum albumins was located or near the tryptophan residues. In the titration with meloxicam there was a small blue shift of maximum emission (λ_{em}) for glycated HSA (about 5 nm), reflecting the predominantly a less polar character of the environment which indicates that the conformation of protein can be affected by the addition of drug. Comparison of influence of meloxicam on the microenvironment of tryptophan residue in subdomain IIA unmodified (native) and glycated HSA is shown in Fig. 2A. Meloxicam could quench about 55% of native HSA fluorescence, and about 40% of glycated HSA. No change of the fluorescence intensity was observed for the control HSA solution over an incubation period.

The quenching process can be usually induced by a collision process or a formation of a complex between quencher and fluorophore. The former is referred to as a dynamic quenching

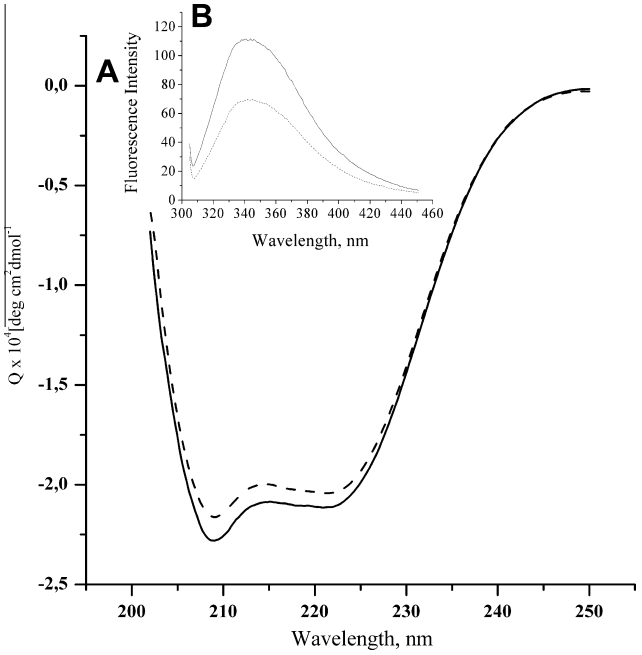


Fig. 1. Effect of glycation on CD (A) and fluorescence (B) spectra of HSA. (—) native HSA (- - - -) glycated HSA. UV CD spectra were taken at protein concentrations 8×10^{-6} M with a 1-mm path length cell. The results were expressed as MRE (mean residue ellipticity) in deg cm^2 dmol^{-1}. Fluorescence spectral changes were measured at protein concentrations 4×10^{-5} M. Excitation at 298 nm.

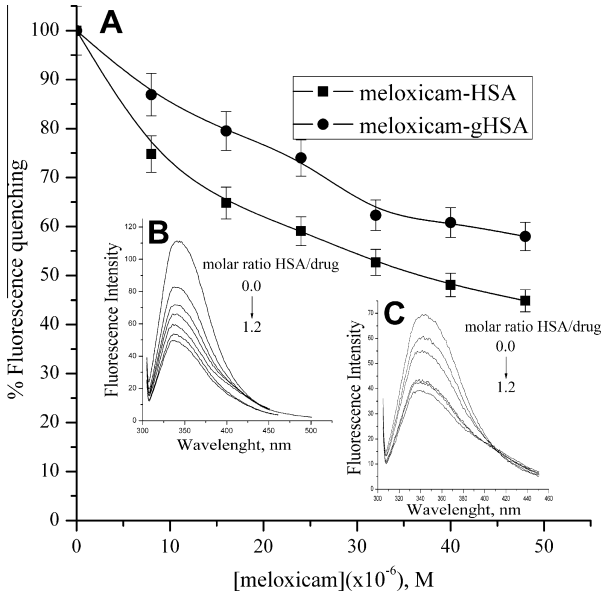


Fig. 2. The comparison of meloxicam binding by native and glycated albumin. Percentage of quenching has been plotted against the molar ratio drugs/HSA (A). Fluorescence spectral changes of unmodified HSA (B) and glycated HSA (C) incubated with meloxicam at various molar ratios, for 24 h at 37 °C. Concentration of HSA: 4×10^{-5} M. Excitation at 298 nm. Molar ratio meloxicam/HSA (gHSA): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2.

mechanism and the latter a static quenching mechanism. Both mechanisms can be distinguished from each other by the differences in temperature dependent behavior.

Stern–Volmer analysis of the interaction between meloxicam and unmodified (native) albumin presented by Seedher and Bhatia et al. [23] indicating on static quenching mechanism.

The interaction between meloxicam and glycated HSA seems to be similar. Stern–Volmer plots were linear and the slope decreased when temperature rose which implied that the quenching mechanism of fluorescence of glycated HSA by meloxicam was most likely a static quenching procedure (data not show).

The calculated association constant (K_a) and the number of binding site (n) for the glycated albumin and meloxicam in two states of excitation, λ_{ex} = 298 and 280 nm were presented in Fig. 3. The binding constants and number of binding sites not showed the great difference in the binding affinity between native and glycated form of albumin. The values of K_a and n for the glycated albumin determined at 310 K are $3.75 \times 10^5\ M^{-1}$ and 1.13 respectively at 280 nm and $3.82 \times 10^5\ M^{-1}$ and 1.11 respectively at λ_{ex} = 298 nm. The association constants of the same order ($10^5\ M^{-1}$) were earlier appointed for native albumin and meloxicam [23]. Furthermore, the values of n approximately equal to 1, which manifested that the existence of just a single binding site in HSA towards meloxicam for both forms.

3.3. Site specific probe

3.3.1. Binding studies in the presence of warfarin

In order to further identify the meloxicam binding sites on HSA, experiments using site markers that specifically bind to a known site or region on HSA molecule have been carried out. Binding location studies between glycated HSA and meloxicam in the presence of two site markers (warfarin and bilirubin) were measured using the fluorescence titration methods and CD spectroscopy. The interaction of ligands with HSA occurs mainly in two regions. Site I has been described as a large hydrophobic cavity present in subdomain IIA. The inside wall of the pocket is formed by hydrophobic side chains, whereas the entrance to the pocket is surrounded by

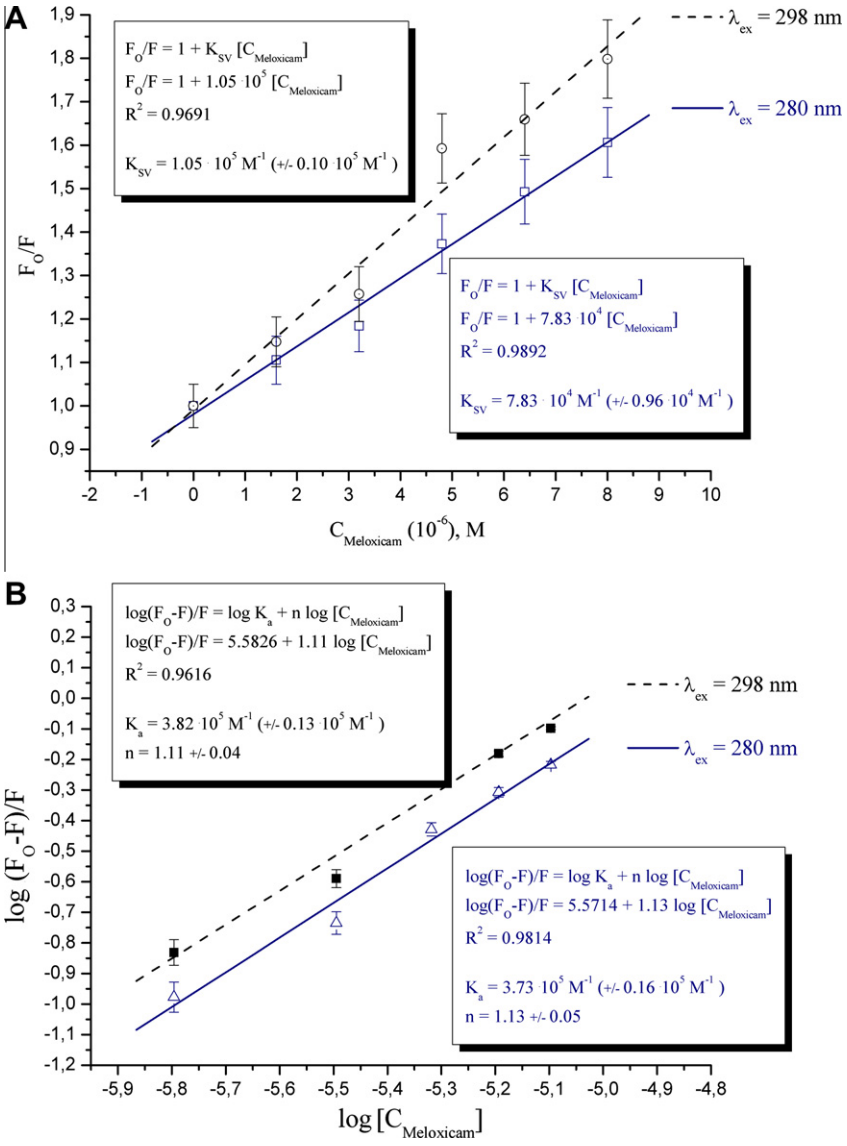


Fig. 3. The Stern–Volmer plots for HSA and gHSA tryptophan quenching caused by meloxicam at 310 K. Concentration of HSA: 4×10^{-5} M. Excitation at 280 and 298 nm. All solutions were prepared at pH 7.4 in 0.05 M sodium phosphate buffer with 0.1 M NaCl (A). Plot of quenching of HSA by meloxicam at 37 °C. The relationship of log $(F_0 - F)/F$ vs. log[meloxicam] (B). The calculated association constant (K_a) and the number of binding site (n) for the glycated and unmodified form of albumin.

positively charged residues Lys 199, His 242, and Arg 257 function sterically and electrostatically in ligand binding. Warfarin (3-(a-acetonylbenzyl)-4-hydroxycoumarin) binds to drug site I with affinity ($K_a \sim 3.43 \times 10^5\ M^{-1}$) [29]. Warfarin shares this binding site with a range of other drugs (including phenylbutazone, tolbutamide, and indomethacin) and thus competes with them for binding to HSA [30].

The affinity of warfarin to albumin was investigated by fluorescence spectroscopy. This method is based on the considerable increase in intrinsic warfarin emission that occurs when the ligand is transferred from a polar, aqueous environment to a hydrophobic binding site on the albumin molecule. Warfarin has a weak fluorescence at 378 nm when excited at 335 nm, and the addition of HSA induced an increase in fluorescence intensity when warfarin binds to a single site in the protein [31]. The interaction of meloxicam with HSA causes a strong inhibition of warfarin binding (about 60%) what indicates a significant modification of warfarin binding site. As is clear from our previous studies [25] the binding site of meloxicam coincides with the binding site of warfarin. The relative fluorescence changes of warfarin with meloxicam treated glycated HSA and native HSA were shown in Fig 4.

The effect of glycation of albumin on the impact of meloxicam on the binding of warfarin was quantitatively studied by determining the percentage of inhibition of bond warfarin after substitution albumin by meloxicam. The percentage of inhibition was calculated using the relationship, $(F - F_{drug})/F \cdot 100\%$ [% inhibition], where F and F_{drug} are the fluorescence intensities of HSA-ligand in the absence and presence of drug, respectively. The percentage inhibition has been plotted against the molar ratio drug/HSA. As shown in Fig. 5 meloxicam binding to glycated albumin only slightly affected the binding of warfarin in contrast to native albumin, where we see very strong inhibition of warfarin bound to protein after meloxicam modification.

As is clear from earlier studies [32] glycation of albumin increased warfarin binding and the association constant what may be entirely attributable to the Lys 199 modification which is in site I. The quantitative differences in the effects of glycation (Fig. 5) determining influence of the meloxicam on the warfarin binding site indicates the significant impact of the glycation on conformation of subdomain IIA and ligand binding properties.

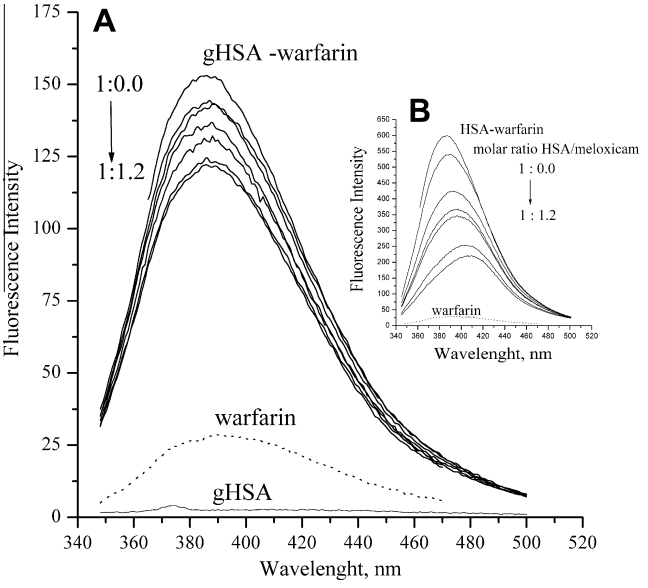


Fig. 4. Relative fluorescence changes of warfarin with meloxicam treated glycated HSA (A) and native HSA (B) excitation at 335 nm. Concentrations of HSA and gHSA were 4×10^{-5} M, the molar ratio of warfarin/albumin 1:1. Meloxicam was incubated 24 h at 37 °C with albumin, before addition of warfarin.

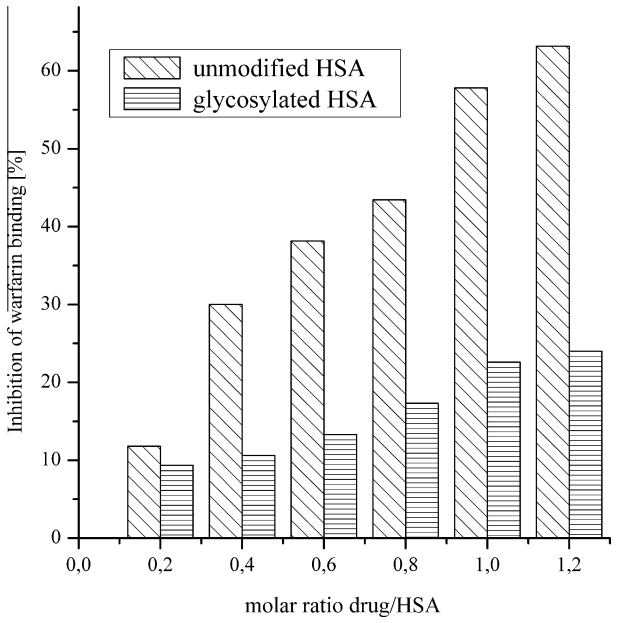


Fig. 5. Effect of meloxicam on fluorescence of warfarin bound to native and glycated form of albumin. Percentage inhibition has been plotted against the molar ratio drug/HSA. Excitation at 335 nm. Meloxicam was incubated 24 h at 37 °C with albumin, before addition of warfarin. The protein concentrations were 4×10^{-5} M, the molar ratio of warfarin/HSA 1:1.

3.3.2. Binding studies in the presence of bilirubin

To provide further information about the binding site of meloxicam to glycated form of albumin the binding of bilirubin as a site I marker was investigated. Bilirubin is the endogenous ligand transported through albumin. Several lines of evidence suggest that high affinity bilirubin binding site on HSA is located in the protein interior corresponding to a hydrophobic pocket in subdomain IIA and several drugs have a strong displacing effect on bilirubin binding [33]. As revealed by circular dichroism spectroscopy that bilirubin binds to HSA in a dissymmetric folded ridge–tile conformation [34]. In this conformation, the bound pigment shows bisignate CD Cotton effects and exhibits greatly increased fluorescence [35]. Previous investigations [25] indicate that meloxicam bound to HSA distinctly modifies the strong binding sites of bilirubin. The magnitude of Cotton effect of the bilirubin with meloxicam modified-HSA decreased significantly (more than 40%) when compared to native protein. As earlier studies have shown bilirubin bound to nonglycated HSA differently than to HSA glycated [36]. The affinity of bilirubin for glycated albumin was about 50% its value for the unmodified nonglycated form.

The results shown in Fig. 6 indicate that Cotton effect of the bilirubin bound to glycated HSA not change significantly, suggesting that glycation did not alter the binding site and type of bond. The affinity of the glycated albumin for bilirubin as for native albumin has been reduced by meloxicam, but observed effect is weaker by half (about 20%) compared with nonglycated albumin.

The obtained results suggest that the modification of albumin by glycation changes the affinity of ligands to the primary binding sites (Sudlow site I). New generation, non-steroidal anti-inflammatory drug, meloxicam affects greatly the affinity to the primary binding sites (Sudlow site I) of albumin, particularly in the area of the binding of warfarin. In contrast to unmodified nonglycated form, the glycation of HSA reduces considerably the influence of meloxicam on the binding of warfarin.

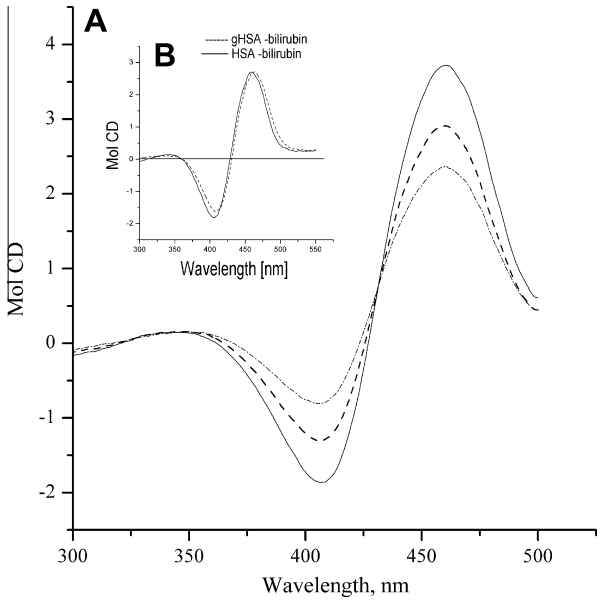


Fig. 6. Effect of meloxicam on the visible CD spectrum of HSA-bilirubin (—), Meloxicam – HSA (– – –), Meloxicam – gHSA (–·–·–). Molar ratio of bilirubin/protein, 1. Concentration of HSA, 8×10^{-5} M, drug – proteins were incubated 24 h at 37 °C at molar ratio 1:1. Mol CD units are dm^{-3} $dmol^{-1}$ cm^{-1} (A). Inset is a visible CD spectra of HSA–bilirubin (—) and gHSA-bilirubin (- - -) (B).

The affinity of the glycated albumin for bilirubin has been reduced how earlier investigations showed [36]. The effect of meloxicam on the binding of bilirubin to glycated albumin is also about half smaller in comparison from nonglycated albumin.

4. Conclusions

In this study, our principal interest was to determine the relative binding affinities of nonglycated and glycated HSA in relation to meloxicam. Therefore, the binding experiments were performed under identical conditions for the two albumin forms.

The previous studies on competitive binding of meloxicam, warfarin and bilirubin to serum albumin [25] pointed to subdomain IIA as the hydrophobic pocket where drug is located.

Both investigated forms are not fatty acid-free. Physiological albumin can be from 0.1 to 2 mol of fatty acids but it seems that this has no significant effect on the conformations of subdomain IIA. There are no essential differences between native (physiological) and defatted albumin in the environment of tryptophan 214. As is clear from previous studies [37] a significant structural changes occur after binding to a larger amount of fatty acids (more than 4 mol). Drugs site I located in subdomain IIA overlaps with low affinity fatty acid site 7, which can be occupied only when the concentration of fatty acids is very high.

In the circulation, HSA becomes glycated by reducing sugars, and the reference range in normal humans is 6–10% glycoalbumin, however, this proportion typically increases to between 20% and 30% in hyperglycemic patients. The principal site of glycation of HSA is Lys-525, but the lysine residues in positions 199, 281 and 439 are also susceptible to glycation. It is known that the effect of glycation on structure, function and metabolism of HSA is primarily caused by blockage of positively charged amino acid residues. The fact that many of these glycation-related modifications are located at or near known drug binding sites on HSA explains why some differences have been previously noted in the binding of certain drugs to normal vs. glycated HSA.

The results of the present study show that glycation process modulated changes on the microenvironment of the binding site influence the binding between meloxicam and HSA and change impact of meloxicam on the binding of other ligands such as warfarin and bilirubin. The affinity of the glycated albumin for bilirubin as for native albumin has been reduced by meloxicam, but observed effect is weaker by half (about 20%) compared with nonglycated albumin. In contrast to native albumin, meloxicam binding to glycated form of the protein only slightly affected the binding of warfarin. It seems possible that the effects on warfarin binding may be entirely attributable to the Lys 199 modification which is in site I.

References

[1] U. Kragh-Hansen, V.T. Chuang, M. Otagiri, Biol. Pharm. Bull. 25 (2002) 695–704.
[2] H. Olsen, A. Andersen, A. Nordbø, U.E. Kongsgaard, O.P. Børmer, BMC Clin. Pharmacol. 4 (2004) 1–9.
[3] T. Peters Jr., All About Albumin: Biochemistry, Genetics, and Medical Application, Academic Press, San Diego, 1996.
[4] D.C. Carter, J.X. Ho, Adv. Protein Chem. 45 (1994) 153–203.
[5] G. Sudlow, D.J. Birkett, D.N. Wade, Mol. Pharmacol. 11 (1975) 824–832.
[6] X.M. He, D.C. Carter, Nature 358 (1992) 209–215.
[7] G. Sudlow, D.J. Birkett, D.N. Wade, Mol. Pharmacol. 12 (1976) 1052–1061.
[8] P. Ulrich, A. Cerami, Recent Prog. Horm. Res. 56 (2001) 1–22.
[9] E. Bourdon, N. Loreau, D. Blache, FASEB J. 13 (1999) 233–244.
[10] K.A. Mereish, H. Rosenberg, J. Cobby, J. Pharm. Sci. 71 (1982) 235–238.
[11] G. Gatti, F. Crema, G. Attardo-Parrinello, P. Fratino, F. Aguzzi, E. Perucca, Ther. Drug Monit. 9 (1987) 389–391.
[12] G. Dannhardt, W. Kiefer, Eur. J. Med. Chem. 36 (2001) 109–126.
[13] J.M.A. Laird, J.F. Herrero, P. Garcia de la Rubia, F. Cervero, Inflamm. Res. 46 (1997) 203–210.
[14] D. Turck, W. Roth, U. Busch, Brit. J. Rheumatol. 35 (1996) 13–16.
[15] R. Banerjee, M. Sarkar, J. Lumin. 99 (2002) 255–263.
[16] A.K. Singla, M. Chawla, A. Singh, J. Pharm. Pharmacol. 52 (2000) 467–486.
[17] M. Stolina, S. Sharma, Y. Lin, M. Dohadwala, B. Gardner, J. Luo, L. Zhu, M. Kronenberg, P.W. Miller, J. Portanova, J.C. Lee, S.M. Dubinett, J. Immunol. 164 (2000) 361–370.
[18] M.L. Parrett, R.E. Harris, F.S. Joarder, M.S. Ross, K.P. Clausen, F.M. Robertson, Int. J. Oncol. 10 (1997) 503–507.
[19] S. Gupta, M. Srivastava, N. Ahmad, D.G. Bostwick, H. Mukhtar, Prostate 42 (2000) 73–78.
[20] N.M. Davies, N.M. Skjodt, Clin. Pharmacokinet. 36 (1999) 115–119.
[21] P. Jolliet, N. Simon, F. Bree, S. Urien, A. Pagliara, P.A. Carrupt, B. Testa, J.P. Tillement, Pharm. Res. 14 (1997) 650–656.
[22] J-W. Bae, M.-J. Kim, Ch-G. Jang, S-Y. Lee, J. Chromatogr. B 859 (2007) 69–73.
[23] N. Seedher, S. Bhatia, J. Pharm. Biomed. Anal. 39 (2005) 257–262.
[24] N. Seedher, S. Bhatia, B. Singh, Chem. Biol. Drug Des. 72 (2008) 297–302.
[25] L. Trynda-Lemiesz, K. Wiglusz, J. Pharm. Biomed. Anal. 52 (2010) 300–304.
[26] G.H. Beaven, S.H. Chen, A. d'Albis, W.B. Gratzer, Eur. J. Biochem. 41 (1974) 539–546.
[27] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, third ed., Springer, New York, 2006.
[28] D. L Mendez, R.A. Jensen, L.A. McElroy, J.M. Pena, R.M. Esquerra, Arch. Biochem. Biophys. 444 (2005) 92–99.
[29] K. Yamasaki, T. Maruyama, U. Kragh-Hansen, M. Otagiri, Biochim. Biophys. Acta 1295 (1996) 147–157.
[30] S. Baroni, M. Mattu, A. Vannini, R. Cipollone, S. Aime, P. Ascenzi, M. Fasano, Eur. J. Biochem. 268 (2001) 6214–6220.
[31] O.J.M. Bos, J.P.M. Remijn, M.J.E. Fisher, J. Wilting, L.H.M. Janssen, Biochem. Pharmacol. 37 (1988) 3905–3909.
[32] K. Nakajou, H. Watanabe, U. Kragh-Hansen, T. Maruyama, M. Otagiri, Biochim. Biophys. Acta 1623 (2003) 88–97.
[33] C.E. Petersen, C.E. Ha, K. Harohalli, J.B. Feix, N.V. Bhagaven, J. Biol. Chem. 275 (2000) 20985–20995.
[34] D.A. Lightner, M. Reisinger, G.L. Landen, J. Biol. Chem. 261 (1986) 6034–6038.
[35] R.F. Chen, Arch. Biochem. Biophys. 160 (1974) 106–112.
[36] N. Shaklai, R.L. Garlick, H.F. Bunn, J. Biol. Chem. 259 (1984) 3812–3817.
[37] J.R. Simard, P.A. Zunszain, J.A. Hamilton, S. Curry, J. Mol. Biol. 361 (2006) 336–351.