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Abacavir and warfarin modulate allosterically kinetics of NO dissociation from ferrous nitrosylated human serum heme-albumin

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Abstract

Human serum albumin (HSA) participates to heme scavenging, in turn HSA–heme binds gaseous diatomic ligands at the heme–Fe-atom. Here, the effect of abacavir and warfarin on denitrosylation kinetics of HSA–heme–Fe(II)–NO (i.e., k_{off}) is reported. In the absence of drugs, the value of k_{off} is $(1.3 \pm 0.2) \times 10^{-4}$ s^{-1}. Abacavir and warfarin facilitate NO dissociation from HSA–heme–Fe(II)–NO, the k_{off} value increases to $(8.6 \pm 0.9) \times 10^{-4}$ s^{-1}. From the dependence of k_{off} on the drug concentration, values of the dissociation equilibrium constant for the abacavir and warfarin binding to HSA–heme–Fe(II)–NO (i.e., $K = (1.2 \pm 0.2) \times 10^{-3}$ M and $(6.2 \pm 0.7) \times 10^{-5}$ M, respectively) were determined. The increase of k_{off} values reflects the stabilization of the basic form of HSA–heme–Fe by ligands (e.g., abacavir and warfarin) that bind to Sudlow's site I. This event parallels the stabilization of the six-coordinate derivative of the HSA–heme–Fe(II)–NO atom. Present data highlight the allosteric modulation of HSA–heme–Fe(II) reactivity by heterotropic effectors.

Keywords: Ferrous nitrosylated human serum heme-albumin; Drug-dependent denitrosylation kinetics; Abacavir; Warfarin; Allostery

Human serum albumin (HSA), the most prominent protein in plasma, provides a depot and carrier for many compounds, affects pharmacokinetics of many drugs, holds some ligands in a strained orientation providing their metabolic modification, renders harmless potential toxins transporting them to disposal sites, accounts for most of the antioxidant capacity of human serum, and displays (pseudo-)enzymatic properties [1–15].

HSA displays a three-domain modular structure probably arising from duplication(s) and divergent evolution of an ancestral gene followed by a fusion event(s). Terminal regions of sequential domains contribute to the formation of flexible interdomain helices linking domain I to II, and II to III, respectively. Each domain consists of two separate sub-domains (named A and B) connected by a random coil [2,3,5,11,13,16,17].

HSA provides a variety of inter-domain and intra-domain ligand binding sites. Heme binds with $K_d = 1.0 \times 10^{-7}$ M to a site located in subdomain IB, with the tetrapyrrole ring arranged in a D-shaped cavity limited by Tyr138 and Tyr161 that provide π–π stacking interaction with the porphyrin and supply a donor oxygen (from Tyr161) coordinating the heme iron. Heme propionates point towards the interface between domains I and III and are stabilized by salt bridges with His146 and Lys190

Abbreviations: Hb, hemoglobin; HPX, hemopexin; HSA, human serum albumin; Lb, leghemoglobin; Mb, myoglobin; Ngb, neuroglobin; sGC, soluble guanylyl cyclase; trHbO, truncated Hb O; IHP, inositol hexakisphosphate; 1-MeIm, 1-methylimidazole.

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[18,19]. Bulky heterocyclic molecules bind preferentially to Sudlow's site I, whereas Sudlow's site II is preferred by aromatic carboxylates with an extended conformation [1–15,20].

The heme pocket and Sudlow's site I are spectroscopically and functionally coupled, indeed Sudlow's site I ligands affect allosterically heme binding and vice versa. Heme binding to HSA inhibits ligand association to Sudlow's site I by stabilizing the basic (B) state of HSA, whereas ligand association to Sudlow's site I impairs human serum heme-albumin (HSA–heme) formation by stabilizing the neutral (N) state of HSA [9–13,21–23]. Interestingly, HSA–heme binds NO and CO and exhibits catalase and peroxidase activity [11,20,24–30]. Furthermore, HSA–heme mutants have been proposed as O_2-carriers [31,32]. Remarkably, abacavir modulates allosterically kinetics of peroxynitrite-mediated oxidation of human ferrous nitrosylated HSA–heme (HSA–heme–Fe(II)–NO) [33].

Here, the effect of abacavir (an anti-retroviral drug) and warfarin (an anticoagulant medication) on denitrosylation kinetics of HSA–heme–Fe(II)–NO is reported. Abacavir and warfarin accelerate NO dissociation from HSA–heme–Fe(II)–NO, highlighting the allosteric modulation of HSA–heme–Fe(II) reactivity by heterotropic effectors which appears to be linked to the redox state of the HSA–heme–Fe atom.

Materials and methods

HSA (⩾96%, essentially fatty acid free), hemin (protoporphyrin IX-Fe(III)) chloride, and warfarin were obtained from Sigma–Aldrich (St. Louis, MO, USA). Abacavir was obtained from Glaxo Wellcome (London, UK). NO (from Aldrich Chemical Co., Milwaukee, WI, USA) was purified by flowing it through an NaOH column in order to remove acidic nitrogen oxides. CO was purchased from Linde AG (Höllriegelskreuth, Germany).

HSA–heme–Fe(II) (7.6×10^{-6} M) was prepared by adding a 1.2-molar excess of HSA to the heme–Fe(II) solution (1.0×10^{-1} M sodium phosphate buffer, pH 7.0) at 10.0 °C. HSA–heme–Fe(II)–NO (3.8×10^{-6} M) was obtained, under anaerobic conditions, by blowing purified NO over the ferrous heme–protein solution (1.0×10^{-1} M sodium phosphate buffer, pH 7.0) at 10.0 °C. Then, the excess of NO was pumped off gently before recording kinetics [20,25,27,29].

The warfarin stock solution (2.0×10^{-2} M) was prepared by dissolving the drug in water at pH 10.0, then adjusting pH to 7.0 with HCl [34]. The abacavir stock solution (1.0×10^{-2} M) was prepared by dissolving the drug in methanol [35]. Drug stock solutions were then mixed with the HSA–heme–Fe(II)–NO (final concentration, 3.8×10^{-6} M) solution to obtain the desired final abacavir and warfarin concentration, ranging between 1.0×10^{-5} M and 5.0×10^{-3} M.

The CO solution was prepared by keeping in a closed vessel the 1.0×10^{-1} M phosphate buffer solution (pH 7.0) under CO at $P = 760.0$ mm Hg anaerobically ($T = 20.0$ °C).

Values of the first-order rate constant for NO dissociation from HSA–heme–Fe(II)–NO (i.e., for NO replacement by CO; k_{off}) were obtained by mixing the HSA–heme–Fe(II)–NO (final concentration, 3.8×10^{-6} M) solution with the CO (final concentration, 1.0×10^{-4} M to 5.0×10^{-4} M) dithionite (final concentration, 1.0×10^{-2} M) solution under anaerobic conditions, at pH 7.0 (1.0×10^{-1} M phosphate buffer) and $T = 20.0$ °C [36], in the absence and presence of abacavir and warfarin (final concentration, 1.0×10^{-5} M to 5.0×10^{-3} M). Kinetics was monitored between 360 nm and 460 nm (wavelength interval = 5 nm). Spectra were collected every 30 s.

$$\text{HSA-heme-Fe(II)-NO} + \text{CO} \xrightarrow{k_{off}} \text{HSA-heme-Fe(II)-CO} + \text{NO}$$

Scheme 1.

The time course for HSA–heme–Fe(II)–NO denitrosylation was fitted to a single exponential process according to the minimum reaction mechanism represented by Scheme 1 [36].

Values of k_{off} were determined from data analysis according to Eq. (1) [37]:

$$[\text{HSA–heme–Fe(II)–NO}]_t = [\text{HSA–heme–Fe(II)–NO}]_i \times e^{-k_{off} \times t} \quad (1)$$

Values of the dissociation equilibrium constant for drug binding to HSA–heme–Fe(II)–NO (i.e., K) were obtained from the dependence of k_{off} on the abacavir and warfarin concentration (i.e., [drug]). Values of K were determined from data analysis, according to Eq. (2) [37]:

$$k_{obs} = k_{off}^{*} \times [\text{drug}]/(K + [\text{drug}]) + k_{off}^{+} \times K/(K + [\text{drug}]) \quad (2)$$

where k_{off}^{*} is the k_{obs} value obtained in the presence of saturating amounts of abacavir or warfarin (i.e., under conditions where [drug] >> K), and k_{off}^{+} is the k_{obs} value obtained in the absence of drugs (i.e., under conditions where [drug] = 0).

Results and discussion

Under all the experimental conditions, the time course for NO dissociation from HSA–heme–Fe(II)–NO conforms to a single-exponential decay for more than 90% of its course, in the absence and presence of abacavir and warfarin (Fig. 1). Values of the first-order rate constant for NO dissociation from HSA–heme–Fe(II)–NO (i.e., k_{off}) are wavelength- and [CO]-independent in the presence of dithionite excess (data not shown).

Values of k_{off} for HSA–heme–Fe(II)–NO denitrosylation increase from $(1.3 \pm 2) \times 10^{-4}$ s^{-1}, in the absence of drugs (i.e., k_{off}^{+} in Eq. (2)), to $(8.6 \pm 0.9) \times 10^{-4}$ s^{-1}, in the presence of saturating amounts of abacavir and warfarin (i.e., k_{off}^{*} in Eq. (2)) (Fig. 1 and Table 1). This finding reflects the stabilization of the B state of HSA–heme–Fe by ligands (e.g., abacavir and warfarin) that bind to Sudlow's site I [9–13,21–23]. This event is accompanied by the stabilization of the six-coordinate derivative of the HSA–heme–Fe(II)–NO species, which is instead predominantly five-coordinated in the N state (i.e., in the absence of ligands of Sudlow's site I) [11,20,27,29].

The 6.6-fold increase of the k_{off} value for NO dissociation from HSA–heme–Fe(II)–NO upon stabilization of the six-coordinated B state by abacavir and warfarin (present study) reflects a behavior similar to that reported for the heme–Fe(II)–NO model compound, where the k_{off} value for NO dissociation increases by 1400-folds following the *trans* binding of 1-methyl-imidazole (1-MeIm) [25] (Table 1). However, the binding in *trans* of a sixth axial ligand to several ferrous nitrosylated hemoproteins is instead generally accompanied by a decrease of the NO dissociation rate constant. Thus, k_{off} values for NO dissociation from five-coordinate ferrous nitrosylated hemopro-