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Cyclodextrin increases the effectiveness of albumin's interaction with antibacterial drugs and their combinations

Anna A. Skuredina* and Elena V. Kudryashova

Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation.
E-mail: anna.skuredina@yandex.ru

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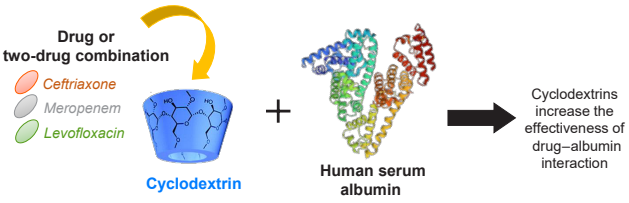
Fluorescence studies indicate that a two-drug combination demonstrates the synergism in binding to human serum albumin if drugs prefer different protein binding sites. The formation of a drug complex with methylated β-cyclodextrin leads to an increase in albumin's quenching and drug binding affinity, while the fluorescence quenching mechanism remains unchanged.



Keywords: human serum albumin, cyclodextrin, antibacterial drug, non-covalent complex, fluorescence.

Respiratory diseases remain a serious social problem demonstrating high mortality worldwide. Bacterial chronic and co-infections require complex therapy and strict drug regiments to achieve effective treatment.[1,2] Despite the rapid development of new drugs and their clinical trials, the application of well-known available remedies is still more reasonable. Nevertheless, drug delivery systems are proposed to increase the therapy efficacy. The encapsulation of drugs into a carrier helps to overcome side effects and rapid excretion of bioactive molecules as well as to increase their solubility, bioactivity, chemical and biological stability, *etc*.[3] Among different types of drug carriers, oligosaccharides cyclodextrins (CD) are frequently favorable for their ability to form host–guest non-covalent complexes with lipophilic molecules. The formation of such complexes alters the physical, chemical and biological properties of the guest.[4,5]

Upon intravenous injection, the drug interacts with different blood components, mainly proteins. Human serum albumin (HSA) is the major plasma protein that transports small compounds of various chemical nature. The understanding of drug–HSA interaction is crucial to predict *in vivo* action of the drug and, as a consequence, its therapeutic effect.[6,7] Sometimes the treatment of respiratory infections requires the use of two and more different drugs; therefore, we are interested in the HSA binding affinity to the drug combinations *via* simultaneous administration.

Here we study the effectiveness of HSA interaction with antibacterial drugs of different classes: ceftriaxone (CT), meropenem (MP), levofloxacin (LV), and their combinations. Furthermore, we highlight the influence of preliminary formation of drug–methyl-β-cyclodextrin (MCD) complex on this interaction.† Figure 1 presents the chemical structures of the compounds.

The investigation of drug–HSA systems was conducted by protein fluorescence quenching analyzed by the Stern–Volmer equation to obtain the Stern–Volmer constant (K_{SV}) and binding affinity constant (K_b).[8,9]

HSA demonstrates an emission peak (λ_{ex} = 280 nm) at 345 nm (Figure 2).[10] In the presence of drug molecules or drug combinations, we observed a pronounced fluorescence quenching. The effect is enhanced with an increase in drug concentration.

According to the calculated quenching rate constants (k_q), only static quenching is observed for all samples under

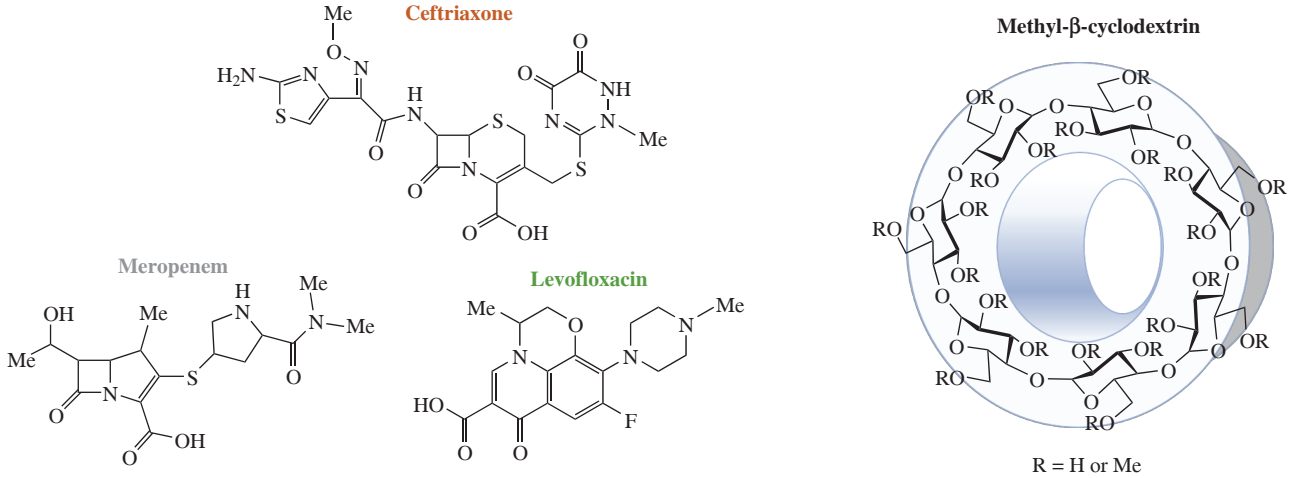


Figure 1 Chemical structures of ceftriaxone, meropenem, levofloxacin, and methyl-β-cyclodextrin. The degree of methyl substitution in methyl-β-cyclodextrin is 1.5–2.1 per one D-glucopyranose unit.

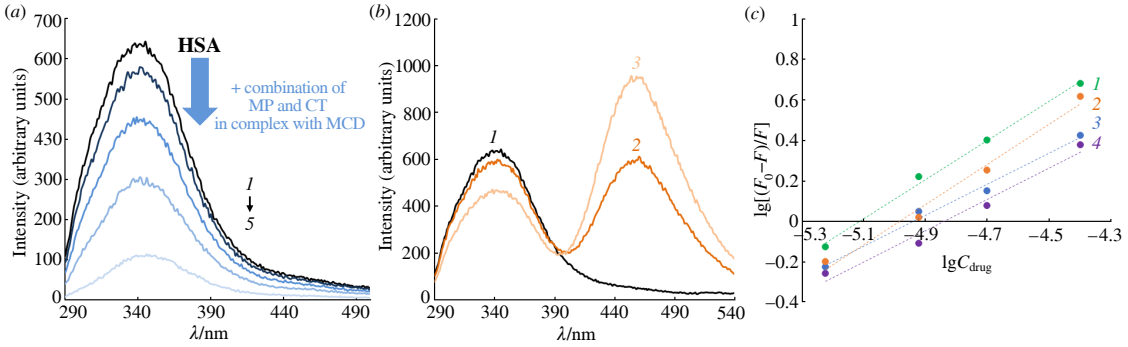


Figure 2 Emission spectra of (*a*) (*1*) HSA and (*2*)–(*5*) HSA–(MP–MCD + CT–MCD) at the molar ratio MP : CT : MCD = 1 : 1 : 2 with an increase in the total drug excess from 0.5 to 3; (*b*) (*1*) HSA, (*2*) HSA–(LV+MP) at the 1 : 0.5 : 0.5 molar ratio, and (*3*) HSA–(LV+MP) at the 1 : 1 : 1 molar ratio, λ_{ex} = 280 nm, pH 7.4, and 37 °C; and (*c*) linearization of HSA quenching curves for (*1*) LV+CT, (*4*) LV+MP and (*2*), (*3*) their complexes with MCD, C_{HSA} = 0.02 mmol dm^{-3}.

Table 1 K_{SV} and K_b values for HSA + drug(s) or its (their) complex with MCD, sodium-phosphate buffer solution, pH 7.4, 37 °C.

HSA+	$K_{SV} \times 10^4$/mol^{-1} dm^3		K_b/mol^{-1} dm^3	
	drug	drug–MCD complex	drug	drug–MCD complex
LV	3.4 ± 0.2	3.1 ± 0.1	(9.9 ± 0.3) × 10^5	(8.9 ± 0.2) × 10^5
MP	0.57 ± 0.03	0.93 ± 0.02	(1.1 ± 0.1) × 10^2	(9.7 ± 0.1) × 10^3
CT	3.8 ± 0.1	4.9 ± 0.2	(1.7 ± 0.1) × 10^5	(3.3 ± 0.3) × 10^5
LV + MP	5.9 ± 0.2	6.5 ± 0.2	(5.6 ± 0.2) × 10^4	(6.1 ± 0.3) × 10^4
LV + CT	10.4 ± 0.2	11.7 ± 0.3	(6.6 ± 0.3) × 10^4	(8.9 ± 0.2) × 10^4
MP + CT	4.8 ± 0.2	7.0 ± 0.2	(2.6 ± 0.3) × 10^5	(4.7 ± 0.3) × 10^5

investigation.[11,12] We found that the presence of neither the second drug molecule nor MCD changes the mechanism of HSA fluorescence quenching.

The estimated Stern–Volmer constants (K_{SV}) for combinations LV + CT and LV + MP exceed the sum of K_{SV} for individual drugs by 20–30%, while a less magnificent effect is observed for CT + MP (Table 1). The synergism might be associated with the pronounced conformational changes in albumin after the interaction with more than one drug simultaneously. Since LV is a more hydrophobic compound than CT and MP, the most pronounced quenching is observed precisely for the combinations with LV that possess higher affinity to the Trp-214 residue in HSA.

HSA has two main drug binding sites, namely site I (subdomain IIA) and site II (subdomain IIIA). CT binds primarily to site I,[13] MT binds to site II,[14] whereas LV does not possess selectivity.[15] We expected that K_b values would demonstrate the same trend as K_{SV} due to the different HSA binding preferences of drugs; *i.e.*, CT and MP could maintain their preference, while LV could demonstrate more preference for the remaining site. Indeed, in the case of MP + CT, the supposed effect is confirmed. However, we observed a pronounced decrease in K_b values for LV + CT and LV + MP compared to the sum of individual values. This effect might be related to the competition of LV with CT or MP for the binding sites. Due to the less binding affinity to protein, we suppose that for LV in combination with CT and MP the most drug molecules will exist in free form in the blood stream. That could lead to fast drug excretion.

HSA adsorbs on large drug carriers forming shells.[16] In contrast, CD interacts with albumin weakly (K_b ~10^2 mol^{-1} dm^3) on protein surface and does not induce magnificent quenching.[17] The formation of the drug–MCD complex demonstrates the strong impact on the binding affinity only for the hydrophilic drugs (Table 1). The binding efficiency of the LV–MCD complex is stronger than that of CT–MCD,[18] which means that CT is more available for binding to HSA than LV. Furthermore, due to MCDs adsorption on the protein surface, the oligosaccharide might act like a 'cap' and prevent the drug's dissociation from albumin. Interestingly, MP–MCD demonstrates the most magnificent increase in binding affinity (100 times) compared to drug–HSA. Hence, the application of drug–MCD might induce a sustained release of drug molecules in the blood due to more efficient binding to HSA.

For combinations of the drug–MCD complex, we observed even more interesting data: an increase in K_b and K_{SV} values up to 40% compared to (drug$_1$ + drug$_2$)–HSA systems. The strongest effect is observed in the case of the MCD complex with MP and CT. Thus, the presence of MCD might lead to pronounced changes in drug biodistribution and pharmacokinetic profiles in the case of two-drug combination therapy. The increase in the binding efficiency might prolong the drug circulation in plasma and, therefore, prolong the antibacterial effect *in vivo*.

In summary, we investigated the interaction of HSA with drug pairs of three different classes that are used in the treatment of respiratory infections. Drug combinations affect albumin fluorescence quenching and decrease the binding affinity possibly due to the competition for binding sites in protein. The formation of drug–MCD complexes leads to an increase in K_{SV} and K_b values for drugs in free forms and their combinations. We observed the major effect for MP that initially demonstrated poor protein binding. The findings reveal the possible decrease in the free drug concentration in blood and, therefore, prolonged the drug circulation in the form of the drug–MCD complex.

The work was performed using a FTIR spectrometer Bruker Tensor 27 and a FTIR microscope MICRAN-3, a Jasco J-815 CD spectrometer, and an AFM microscope NTEGRA II within the framework of the Program Development of the Moscow State University.

† C_{HSA} was maintained 0.02 mmol dm^{-3} (PBS, pH 7.4) in all experiments, while the molar excess of drug or equimolar drug combination was varied from 0.5 to 3. In the case of the MCD complex with a drug molecule, the samples with HSA were obtained similarly.

The drug–MCD complex was prepared by mixing solutions (drug/MCD molar ratio remained 1:1) and was incubated at 37 °C for 20 min before adding to HSA.

All systems were incubated at 37 °C for 40 min before the fluorescence measurements (λ_{ex} = 280 nm) in the range of 290–500 nm using a Varian Cary Eclipse fluorescence spectrometer (Agilent Technologies, USA).

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