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ELUCIDATING THE INFLUENCE OF SOME METAL IONS ON THE BINDING OF ANTIMALARIAL DRUGS TO HUMAN SERUM ALBUMIN: SPECTROSCOPIC APPROACH

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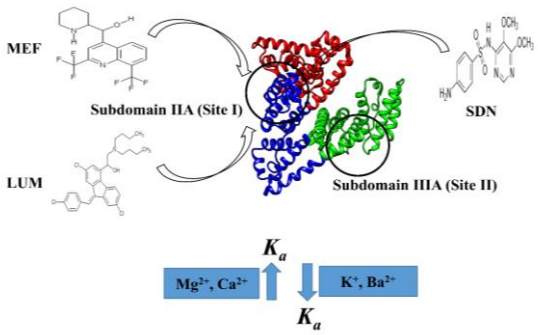
Influence of six metal ions viz. Mg^{2+}, K^{+}, Ca^{2+}, Mn^{2+}, Cu^{2+} and Ba^{2+} on the binding affinity of human serum albumin (HSA) towards three antimalarial drugs, namely, sulfadoxine (SDN), mefloquine (MEF) and lumefantrine (LUM) was investigated using fluorescence quenching titrations. The effects of metal ions (Mn^{2+}, Cu^{2+} and Ba^{2+}) were similar on the affinity of HSA towards SDN, MEF and LUM. Values of the association constant, K_a, for SDN/MEF/LUM−HSA interactions in the presence of these metal ions, decreased significantly in the order: Mn^{2+} > Cu^{2+} > Ba^{2+}. On the other hand, the presence of Mg^{2+}, K^{+}, and Ca^{2+} affected the binding affinity for these drugs differently. Whereas a slight increase in the K_a value of the SDN−HSA complex was seen in the presence of Mg^{2+}, Ca^{2+} produced a similar effect on the LUM−HSA system. For MEF−HSA and LUM−HSA systems, effects of K^{+} and Mg^{2+} were similar, showing a decrease in the K_a value, being more effective with K^{+}; however, this decrease was more significant than Mn^{2+}, Cu^{2+} and Ba^{2+}. Values of *'n'* remained approximately equal to 1, hinting at a single binding site for each drug.



INTRODUCTION

Metal ions play a vital role in cell functions by participating in many physiological and metabolic processes, such as potassium in cell signalling and maintaining tissue electrolyte balance, magnesium in ion channels, calcium in blood coagulation, manganese in antioxidant defences, copper in mitochondrial respiration, etc. Metal ions also serve as cofactors of many enzymes.[1,2] They also contribute to stabilizing macromolecular structures and their interactions with other molecules.[2-4]

Human serum albumin (HSA), the primary carrier for diverse intrinsic and extrinsic molecules, including drugs in blood plasma, is also known to bind to metal ions, which has a significant role in different pharmaceutical conditions.[5-8] The binding of metal ions to HSA may result in structural changes in the protein, which subsequently affect the half-life, stability

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and level of toxicity in human circulation.[9] Additionally, the binding of drugs to the protein is often influenced or hindered in the presence of metal ions.[10,11]

Recently, we have characterized the binding of three antimalarial drugs, namely sulfadoxine (SDN), mefloquine (MEF) and lumefantrine (LUM) to human serum albumin.[12-14] Given the influence of metal ions on drug-protein interaction, it is of great interest to investigate the effect of some common metal ions on the interaction of these antimalarial drugs with HSA. To achieve this goal, a fluorescence spectroscopic technique was employed in this study.

RESULTS AND DISCUSSION

The fluorescence spectroscopic technique is often used to study the influence of metal ions on the interaction between various drugs and HSA.[16-18] Some common metal ions in the blood plasma might interfere with the drug binding to the plasma proteins.[19] To check if six metal ions (Mg^{2+}, K^{+}, Ca^{2+}, Mn^{2+}, Cu^{2+} and Ba^{2+}) influence antimalarial drug-HSA (SDN-HSA, MEF-HSA and LUM-HSA) interactions, fluorescence quenching titration of HSA with increasing SDN/MEF/LUM concentrations was carried out both in the absence and presence of metal ions in PB7.4 at 298 K. Variation in the fluorescence intensity of HSA at 343 nm ($FI_{343\ nm}$) with increasing SDN concentrations in the absence and presence of these metal ions is shown in Fig. 1A. Presence of metal ions produced effects in terms of SDN-induced quenching of protein fluorescence. Whereas reduced quenching was observed with Ca^{2+}, K^{+}, Mn^{2+}, Cu^{2+} and Ba^{2+} compared to that observed in their absence, Mg^{2+} produced an enhanced quenching effect. The quenching pattern showed the following order in the decreasing trend: $MgCl_2$ > HSA > $CaCl_2$ > KCl > $MnCl_2$ > $CuCl_2$ > $BaCl_2$. Fig. 1B depicts the results obtained from these experiments in terms of relative fluorescence intensity at 343 nm ($FI_{343\ nm}$) against MEF concentration.

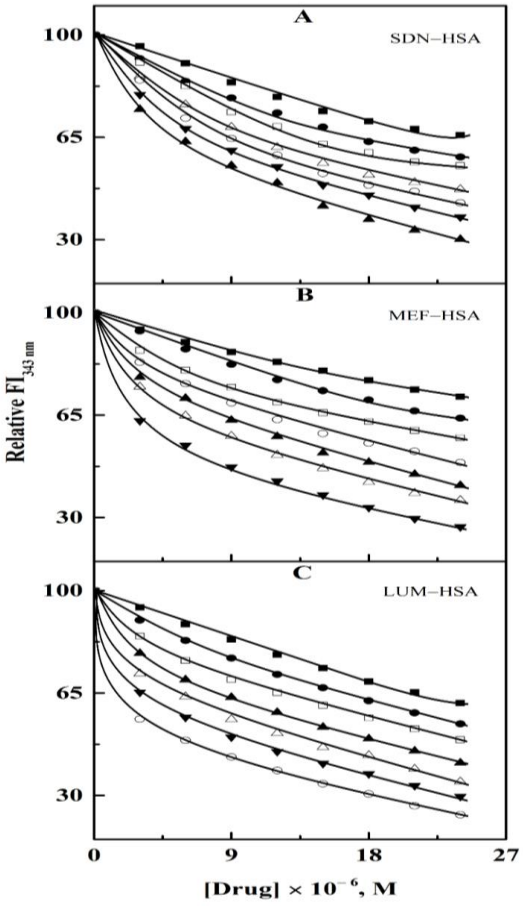

Fig. 1 – Plots showing the decrease in the fluorescence intensity of HSA at 343 nm against increasing drugs' concentrations without and with different metal ions, obtained in PB 7.4 at 298 K. Absence of metal ions (closed triangles, down-pointing); Mg^{2+} (closed triangles, up-pointing); K^{+} (open triangles, up-pointing); Cu^{2+} (closed circles); Ca^{2+} (open circles); Ba^{2+} (closed squares) and Mn^{2+} (open squares).

Although increasing MEF concentrations produced a decrease in the $FI_{343\ nm}$ value in all cases, a lesser decrease was noticed in the presence of metal ions at all MEF concentrations compared to that observed with HSA alone (in the absence of metal ions). As shown in Fig. 1B, reduction in the $FI_{343\ nm}$ value with increasing MEF concentrations followed the trend: HSA > KCl > $MgCl_2$ > $CaCl_2$ > $MnCl_2$ > $CuCl_2$ > $BaCl_2$. Figure 1C shows the influence of these metal ions on the LUM-induced changes in the fluorescence intensity at 343 nm ($FI_{343\ nm}$) of HSA. Except for Ca^{2+}, other metal ions were found to inhibit the LUM-induced quenching of HSA fluorescence to different extents. The quenching pattern followed the order: $CaCl_2$ > HSA > KCl > $MgCl_2$ > $MnCl_2$ > $CuCl_2$ > $BaCl_2$.

To further verify the influence of metal ions on SDN-HSA interaction, fluorescence data were treated according to the double logarithmic equation described in the Materials and Methods section, and the resulting double logarithmic plots are shown in Fig. 2A. The values of K_a and n are presented in Table 1. The K_a values obtained in the presence of metal salts indicated a smaller but significant decrease in the same order as described above. The trend of the influence of metal ions on MEF-HSA interaction was also evident from the K_a values (Table 1) collected from the double logarithmic plots, which are depicted in Fig. 2B. To get better insights about the influence of metal ions on LUM-HSA, fluorescence data were also transformed into double logarithmic plots, which are displayed in Fig. 2C. The K_a values obtained in the presence of metal ions (Table 1) revealed similar trend as described above by showing small but significant decrease with these metal ions except Ca^{2+}, which produced a small increase in the K_a value. The values of n were found to be 0.99 ± 0.02, 0.98 ± 0.01 and 0.99 ± 0.01 at 298 K for SDN−HSA, MEF−HSA and LUM−HSA, respectively, in the absence of metal ions. In the presence of various metal ions, values of n fell within the same range. Being approximately equal to 1.0, it suggests that each drug (SDN, MEF and LUM) binds to only a single binding site on HSA.

The possible reason for the decrease in the K_a value might be a competition between the drug and the metal ions for the same binding site on HSA or the formation of metal ion-drug complexes, which have a lower binding affinity or possible change in the protein conformation.[20,21] Formation of metal ion-drug complexes with higher or lower affinity as well as direct competition or allosteric effects seem to be possible speculations for the metal ion influences on the drug (SDN/MEF/LUM)-HSA interaction.[22,23] The binding of drugs to HSA is often influenced by the presence of metal ions.[11,24] Therefore, the episode of metal ion influence could prolong or shorten the drug's half-life in blood plasma, which may improve or narrow the drug efficacy.[25]

Table 1

Values of the binding constant, K_a and the number of binding sites n for SDN−HSA, MEF−HSA and LUM−HSA interactions in the absence and the presence of different metal ions, 30 μM, as studied in PB 7.4 at 298 K

Metal ion	SDN–HSA		MEF–HSA		LUM–HSA	
	$K_a \times 10^4$ (M^{-1})	n	$K_a \times 10^4$ (M^{-1})	n	$K_a \times 10^4$ (M^{-1})	n
-	3.49 ± 0.02	0.99 ± 0.02	4.67 ± 0.09	0.98 ± 0.01	6.01 ± 0.19	0.99 ± 0.01
Mg^{2+}	4.07 ± 0.19	0.98 ± 0.02	2.32 ± 0.07	0.99 ± 0.01	4.32 ± 0.15	0.98 ± 0.01
K^+	3.29 ± 0.01	0.99 ± 0.002	2.87 ± 0.11	0.99 ± 0.001	5.25 ± 0.13	0.98 ± 0.02
Ca^{2+}	3.41 ± 0.11	0.98 ± 0.003	1.98 ± 0.19	0.98 ± 0.01	7.18 ± 0.04	0.99 ± 0.02
Mn^{2+}	3.17 ± 0.09	0.98 ± 0.01	1.72 ± 0.01	0.98 ± 0.02	3.17 ± 0.08	0.98 ± 0.02
Cu^{2+}	3.09 ± 0.06	0.99 ± 0.01	1.25 ± 0.20	0.99 ± 0.002	2.82 ± 0.10	1.00 ± 0.002
Ba^{2+}	2.53 ± 0.13	0.99 ± 0.001	0.94 ± 0.03	0.99 ± 0.02	1.72 ± 0.08	0.99 ± 0.003

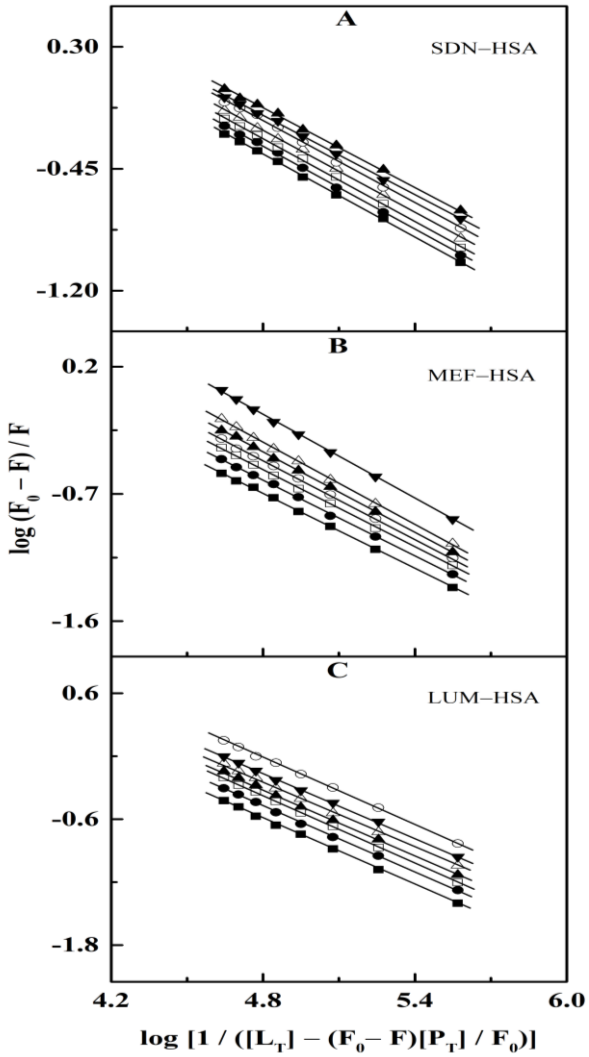

Fig. 2 – Double logarithmic plots for (A) SDN−HSA, (B) MEF−HSA and (C) LUM−HSA systems in the absence and the presence of different metal ions, obtained in PB 7.4 at 298 K. Absence of metal ions (closed triangles, down-pointing); Mg^{2+} (closed triangles, up-pointing); K^{+} (open triangles, up-pointing); Cu^{2+} (closed circles); Ca^{2+} (open circles); Ba^{2+} (closed squares) and Mn^{2+} (open squares).

EXPERIMENTAL

1. Materials

Essentially fatty acid-free human serum albumin (HSA) (purity > 99%; Lot #068K7538V) and sulfadoxine (SDN) were purchased from Sigma Chemical Company (St Louis, MO, USA). Mefloquine (MEF) and lumefantrine (LUM) were supplied by Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA).

Human serum albumin stock solution was prepared in 60 mM sodium phosphate buffer, pH 7.4 (PB 7.4). The protein concentration was determined spectrophotometrically using $\mathcal{E}_m$ of 36, 500 M^{-1} cm^{-1} at 280 nm. SDN, MEF and LUM stock solutions were prepared in dimethyl sulfoxide (DMSO). The stock solutions of chloride salts of various metal ions, namely, KCl, $MgCl_2$, $CaCl_2$, $MnCl_2$, $CuCl_2$ and $BaCl_2$, were prepared using MiliQ and then diluted in PB 7.4 buffer to the specified concentration.

2. Titration experiment and data analysis

The fluorescence quenching titration method was carried out to investigate the effect of metal ions on the binding affinity of HSA towards antimalarial drugs. A fixed volume (0.20 mL) of HSA stock solution (3 μM) was mixed with (0.89 ml) of stock solution (30 μM) of various metal salts, and the mixture was incubated for 30 min. Increasing drug concentrations (3− 24 μM using 3 μM interval) were added making the total volume of 3 mL with PB7.4. An additional incubation for 30 min was employed before the fluorescence scans. The experiments were conducted at ~ 298 K for SDN, MEF and LUM to evaluate the binding constant values (K_a).

The binding constant, K_a values, were obtained by treating the fluorescence data according to the double logarithmic equation:[15]

$$\log (F_0 - F) / F = \mathrm{n} \log K_\mathrm{a} - \mathrm{n} \log [1 / ([L_T] - (F_0 - F)\,[P_T] / F_0)]$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, whereas $[L_T]$ and $[P_T]$ represent the total concentration of the ligand and the protein, respectively.

3. Statistical analysis

All experiments were carried out at least three times and presented with the average values. The curves' processing, fitting and smoothing were made using the OriginPro 2016 software (OriginLab Corp., Northampton, MA).

CONCLUSIONS

Metal ions-induced influence on the interaction between antimalarial drugs (SDN, MEF and LUM) and HSA has been characterized. An increase in the K_a value in the presence of metal ions suggested the formation of the metal ion− drug complex, which may enhance the drug binding to protein. In contrast, a metal ion-induced decrease in the K_a value may likely lead to conformational changes in HSA that might inhibit the drug binding to protein. Of all the six metal ions used in this study, *i.e.*, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Cu^{2+} and Ba^{2+}, a small but significant decrease in the K_a value was noticed except in the presence of Mg^{2+} for SDN−HSA complex, and Ca^{2+} for LUM−HSA complex, which shows an increase in the K_a values. There was a steady small but significant decrease in the K_a value in the presence of these metal ions in the case of the MEF−HSA complex.

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