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Role of the magnesium cation on antihypertensive molecule–human serum albumin binding: affinity chromatography approach

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Abstract

The role of the Mg^{2+} cation on antihypertensive molecule binding on human serum albumin (HSA) was studied by affinity chromatography. The thermodynamic data corresponding to this binding were determined for a wide range of Mg^{2+} concentrations (c). For the nifedipine molecule, an increase in the Mg^{2+} concentration produced a decrease in binding due to a decrease in the electrostatic interactions. For verapamil and diltiazem, which have the highest solvent accessible surface area, the solute binding on HSA was divided into two Mg^{2+} concentration regions. For a low c value below c_c ($\approx$1.6 mmol/l), the binding dependence with c was similar to that of nifedipine. For c above c_c the hydrophobic effect created in the bulk solvent associated with a decrease in the van der Waals interactions between the solute molecule and the HSA implied a decrease in its binding. These results showed that for patients with hypertension, an Mg^{2+} supplementation during treatment with these antihypertensive molecules can increase the active pharmacological molecule concentration.

Keywords: Protein binding; Magnesium cation; Human serum albumin; Nifedipine; Verapamil; Diltiazem

1. Introduction

Magnesium is an important factor in the physiology of the cardiovascular apparatus and the pathogenesis of cardiovascular diseases. In some hypertensive patients a magnesium deficit, because of its numerous noxious actions on the nephrocardiovascular apparatus, must be controlled [1–4] and may sometimes behave as a cofactor of constitutional or acquired hypertensive factors [4]. This seems to be particularly the case with stress sensitive patients with labile hypertension because of the dose links between magnesium deficit and stress [5–7]. The vasodilatator action of parenteral magnesium human serum albumin (HSA) has been well-known for over a century [8]. Hypotensive action of parenteral magnesium may be used in hypertensive patients and particularly in preeclampsia. HSA is the most abundant protein in blood and can reversibly bind a large number of pharmacological substances such as antihypertensive molecules. HSA was the model ligand used in a great number of studies. The main advantage of using HSA is that data are available for its interaction with a wide range of organic and inorganic compounds [9]. Affinity chromatography with

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HSA immobilized on the support is specially suited to the study of drug–protein interactions. The association constants of many ligands have been determined by zonal elution [10] or frontal analysis [11]. The thermodynamic process involved in the binding have already been studied [12–17]. This paper describes a study to investigate each individual factor, the effect of the concentration, c, of Mg^{2+} in the bulk solvent (i.e., the mobile phase) and the column temperature T on the binding process of three antihypertensive drugs with human serum albumin. The shapes of the Van't Hoff plots were used to assess the effects of temperature and Mg^{2+} concentration changes on the binding process. Enthalpy–entropy compensation was also applied to evaluate this binding mechanism.

1.1. Thermodynamic relationships

Solute retention is usually expressed in terms of the retention factor, k', using the well known equations:

$$\ln k' = -\Delta H^0/(RT) + \Delta S^{0}* \quad (1)$$

$$\Delta S^{0}* = \Delta S^0/R + \ln \phi \quad (2)$$

where ΔH^0 and ΔS^0 are, respectively, the enthalpy and entropy changes accompanying solute transfer from the mobile to the HSA-stationary phases, T is the temperature, R the gas constant, and ϕ the column phase ratio (volume of the stationary phase divided by the volume of the mobile phase). A plot of $\ln k'$ against $1/T$ is called a Van't Hoff plot. For a linear plot, the slope and intercept are, respectively, $-\Delta H^0/R$ and $\Delta S^{0}*$. This provides a convenient way of calculating the thermodynamic constants ΔH^0 and ΔS^0 if the phase ratio is known or can be calculated. Although ΔS^0 is not usually provided, because of the ambiguity in the phase ratio for commercial columns, $\Delta S^{0}*$ varies identically with ΔS^0.

2. Experimental

2.1. Apparatus

The high-performance liquid chromatography (HPLC) system consisted of a Merck–Hitachi pump L7100 (Nogent-sur-Marne, France), an Interchim Rheodyne injection valve Model 7125 (Montluçon, France) fitted with a 20-μl sample loop and a Merck L 4500 diode array detector (Nogent-sur-Marne, France). A HSA protein chiral Shandon column (Montluçon, France) (150×4.6 mm) was used with controlled temperature in a Interchim Crococil oven TMN° 701 (Montluçon, France). After each utilization, the column was stored at 4°C until further use. Throughout the study, the flow-rate was maintained constant and equal to 0.7 ml/min.

2.2. Solvent and samples

Sodium hydrogenphosphate and sodium dihydrogenphosphate were supplied by Prolabo (Paris, France). $MgCl_2$ was obtained from Sigma–Aldrich (Saint-Quentin, France). Water was obtained from an Elgastat option water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge. Nifedipine, verapamil and diltiazem, the three antihypertensive drugs, were obtained from RBI (Natick, USA) and were made fresh daily at a concentration of 20 mg/l. The chemical structures of these compounds are given in Fig. 1. Sodium nitrate was used as a dead time marker (Merck). The mobile phase consisted of a sodium phosphate buffer at pH 7.3 (pH of the plasma) with $MgCl_2$ concentrations equal to 0.3, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3.0 mmol/l. It was recently demonstrated that the electrostatic attractions played a major role on the Mg^{2+} binding mechanism on HSA [18–20]. Thus, in order to increase this ionic interaction the phosphate buffer concentration was very low, equal to $7\cdot10^{-4}$ M. A 20-μl volume of each solute was injected and the retention times were measured.

2.3. Temperature study

Retention factors of each solute were determined at six temperatures 20, 25, 30, 35, 40 and 45°C. The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each experiment. To study this equilibration, the retention time of the diltiazem was measured every hour for 5 h and again after 23 and 24 h. The maximum relative difference of the retention time of this compound was always 0.5%, making the chromatographic

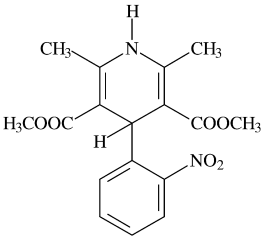
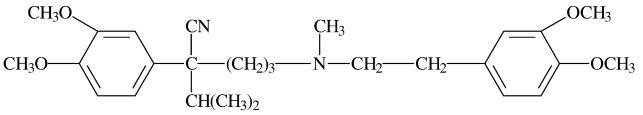
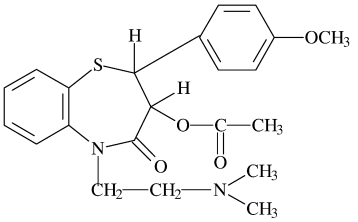
Nomenclature	Chemical structure
nifedipine	
verapamil	
diltiazem	

Fig. 1. The three antihypertensive molecules.

system sufficiently equilibrated for use after 1 h. The antihypertensive molecules were injected three times at each temperature and Mg^{2+} concentration. Once the measurements were completed at the maximum temperature, the column was immediately cooled to ambient conditions to minimize the possibility of any unfolding of the immobilized HSA.

3. Results and discussion

The asymmetry factors [21,22] of all peaks (A_s) calculated from measurements made at 50% of the total peak height were in the range $1.00 \leq A_s \leq 1.22$. The experimental k' values were determined at the maxima of the chromatographic peaks. The k' values were calculated for the $15 \times 6 = 90$ experiments. Each experiment was repeated three times. The relative standard deviations of the k' values were usually less than 0.8%, indicating the high reproducibility and stability of the chromatographic system. The surface s corresponding to $\ln k'$ vs. c and $1/T$ was plotted as a three-dimensional diagram for verapamil (Fig. 2). The retention factor k' was linked to the binding constant K of the drug with HSA by $k' = \phi K$. k' represents the binding intensity. When c increased,

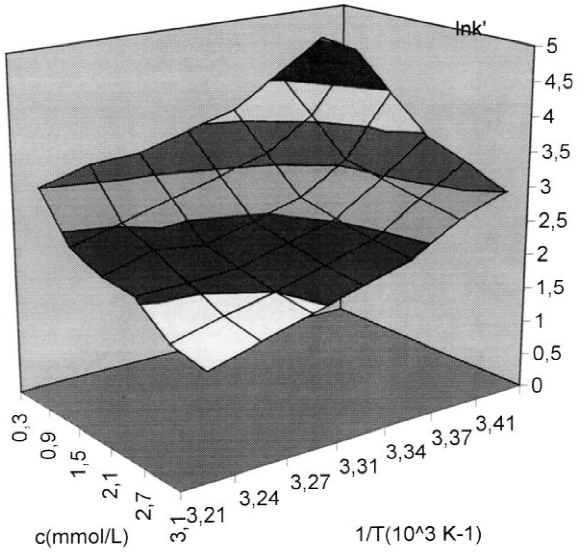


Fig. 2. The response surface of a plot of ln k' values against c and $1/T$ for verapamil.

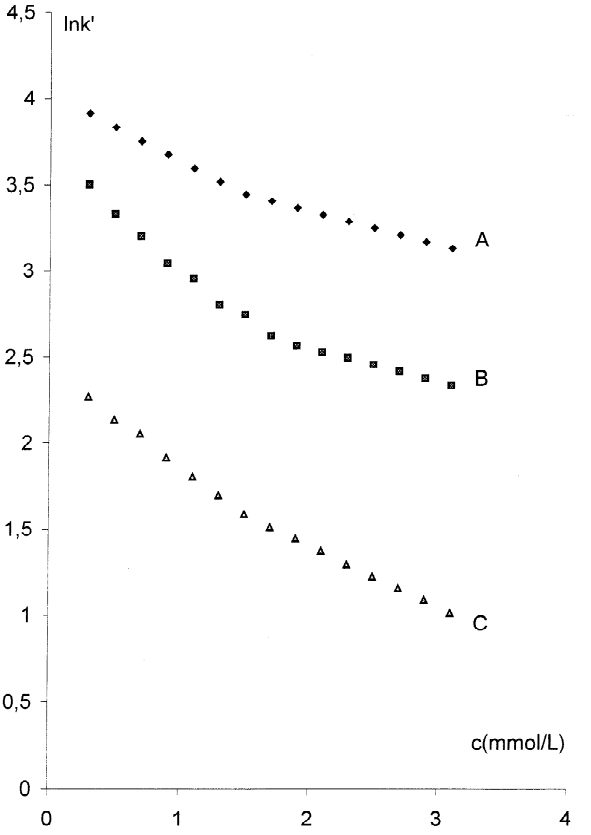


Fig. 3. Plot of ln k' against c for all antihypertensive molecules at a column temperature equal to 25°C: (A) nifedipine, (B) verapamil, (C) diltiazem.

the k' values for the solutes decreased (Fig. 3). This result showed that in the range of the Mg^{2+} concentrations studied, the binding of the antihypertensive molecules decreased as c increased. The Van't Hoff plots of Eq. (1) were linear for all solutes and the correlation coefficient r, for all the fits was over 0.998. Figs. 4 and 5 show the variation in the ΔH^0 and ΔS^{0}*, respectively, with c for the solute molecules. The negative values of the ΔH^0 and ΔS^{0}* terms demonstrate that the binding was controlled enthalpically. This is consistent with results reported in the literature for various chromatographic systems [23,24]. A negative ΔH^0 indicates that it is energetically favorable for the antihypertensive molecules to be bound to HSA. Negative ΔS^{0}* proves the apparently lower degree of freedom of solutes bound with HSA. Figs. 4 and 5 show that ΔH^0 and ΔS^{0}* were smaller for verapamil than for diltiazem. Binding with HSA was, therefore, more ordered and more energetically stabilized with verapamil than with diltiazem. In order to gain further insight into the validity of the binding model, the enthalpy–entropy compensation was examined. This approach has been previously used in chromatographic procedures to analyse and compare the retention mechanism for a group of compounds [16,23–27]. The enthalpy–entropy compensation can be described by the following relation:

$$\Delta H^0 = \beta \Delta S^0 + \Delta G^0_\beta \quad (3)$$

where ΔG^0_β is the Gibbs free energy of a physico–chemical interaction at a compensation temperature β. Eq. (3) shows that if a plot of ΔH^0 against ΔS^{0}* is linear, then the solutes are retained by an essentially identical interaction mechanism. A $\Delta H^0 - \Delta S^{0}$* plot determined at all the different values of c was drawn for the three compounds. The correlation coefficients for the linear fits were equal to 0.95. Fig. 6 shows ΔH^0 values plotted in relation to ΔS^{0}*. This degree of correlation can be considered to be adequate to verify enthalpy–entropy compensation and indicates that the interaction mechanism between

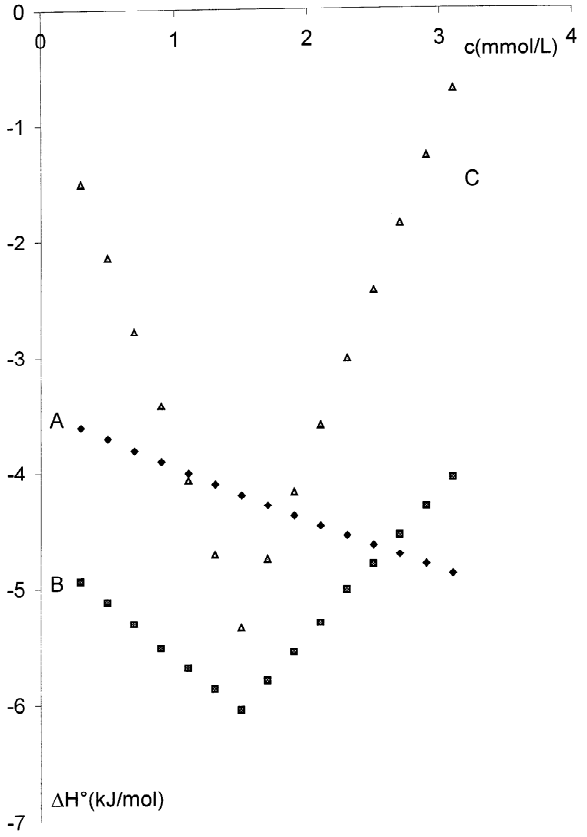


Fig. 4. Plot of ΔH^0 (kJ/mol) against c for the three antihypertensive molecules: (A) nifedipine, (B) verapamil, (C) diltiazem.

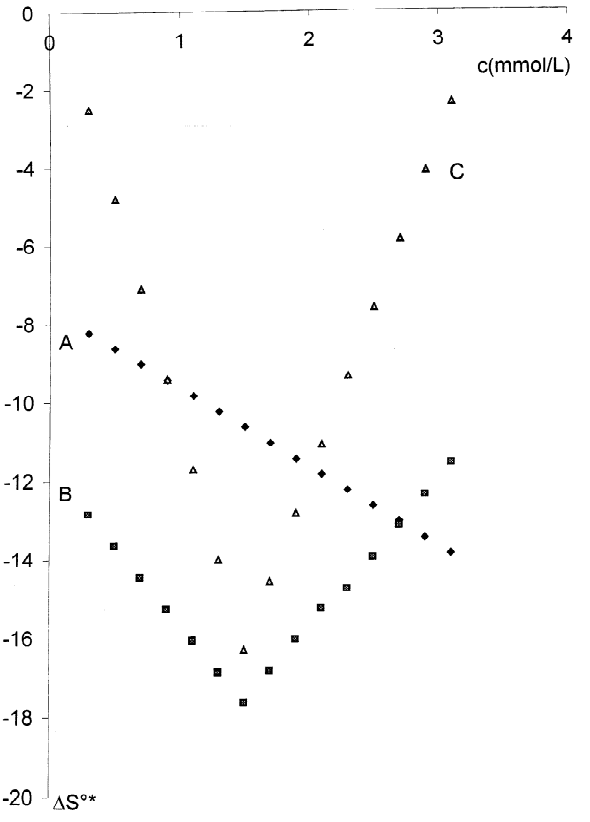


Fig. 5. Plot of ΔS^{0}* (no unit) against c for the three antihypertensive molecules: (A) nifedipine, (B) verapamil, (C) diltiazem.

the solutes and HSA was independent of the Mg^{2+} concentration and identical for the three solutes. Consequently, the binding mechanism with HSA appeared to be identical for the three solutes and the same with or without Mg^{2+} in the bulk solvent. This revealed that the three antihypertensive drugs bound at the same location on HSA and the eventual interactions of the Mg^{2+} cation in this binding location on HSA (at pH 7, HSA was negatively charged [18,28,29]) and the possible complexation of these drugs with Mg^{2+} bound on HSA seemed to be negligible. As can be seen in Figs. 4 and 5, ΔH^0 and ΔS^{0}* became increasingly negative as c was increased over the whole concentration range for nifedipine and below a critical c_c value of approximately 1.6 mmol/l for verapamil and diltiazem. In these low Mg^{2+} concentration ranges, the ionic double layer of the charged species was thick with a high Debye length. The salt concentration increase was responsible for a Debye length reduction by affecting the electrostatic shielding which governed a ionic attraction decrease. It has been known for several years that the interaction between the ionic species in aqueous solution are characterized by small positive enthalpy and entropy changes [30,31]. Thus, the decrease in the antihypertensive drug binding was accompanied by a reduction in enthalpy and entropy changes for the transfer of the drugs from the bulk solvent to HSA. In these domains, as c increased, ΔH^0 and ΔS^{0}* became progressively more negative corresponding to a weaker retention. When $c > c_c$ for verapamil and diltiazem only, ΔH^0 and ΔS^{0}* increased. This can be explained by the fact that these two solutes have the highest accessible surface area (ASA) [32] (Fig. 1). Thus, the osmo-

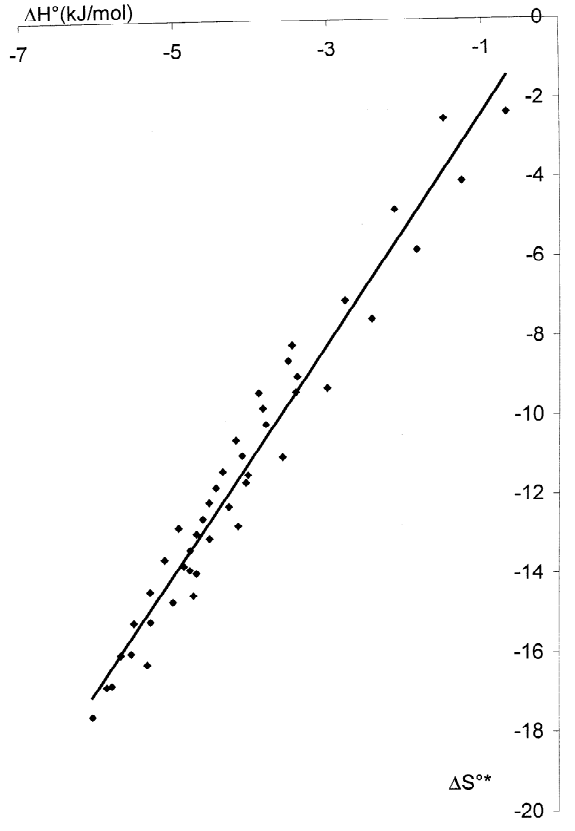


Fig. 6. Enthalpy–entropy compensation represented by the $\Delta H^0 - \Delta S^{0*}$ plot at the different values of Mg^{2+} concentration for the three antihypertensive molecules..

tropic character (i.e., hydrophobic effect) of the Mg^{2+} cation [33–35] which is correlated with the ASA [36] was, therefore, the most important for verapamil and diltiazem. The observed ΔH^0 and ΔS^{0*} variations with c can be interpreted by an increase in the surface tension of the bulk solvent, i.e., the hydrophobic effect. In this domain, as c increased ΔH^0, ΔS^{0*} became progressively less negative corresponding to a weaker retention. It is important to note that in the range of the biological magnesium concentrations in plasma (0.7–0.9 mmol/l), the binding of the three antihypertensive drugs was controlled by the electrostatic interactions. An increase in the Mg^{2+} cation concentration produced an increase in the free drug concentration (not bound with HSA, i.e., the active pharmacological form). However, at a physiological phosphate buffer concentration, such an effect may be drastically reduced. This result corroborates the fact that a magnesium supplement during treatment with these drugs can be useful for patients who are suffering from hypertension.

4. Conclusion

The role of the magnesium cation on the binding mechanism on HSA of three antihypertensive drugs was examined. The dependence of retention data on temperature was investigated and trends in thermodynamic parameters were determined. The results showed that for nifedipine, in the 0.3–3 mmol/l concentration range of Mg^{2+}, an increase in the Mg^{2+} concentration produced a decrease in the electrostatic interactions and thus in the binding affinity. For verapamil and diltiazem, a critical magnesium concentration c_c was observed ($c_c \approx 1.6$ mmol/l). Below c_c, their binding dependence with c on HSA was similar to that of nifedipine. Above c_c for these solutes, i.e., diltiazem and verapamil, which had the highest solvent accessible area, an increase in the Mg^{2+} concentration produced an increase in the hydrophobic effect. This increase in the hydrophobic effect in the bulk solvent associated with a decrease in the vdw interactions between these solutes and HSA explain the binding affinity decrease. These results showed that for patients who suffer from hypertension, an Mg^{2+} supplement can increase the active pharmacological drug concentration (even if at a physiological phosphate buffer concentration, a such effect may be drastically reduced) and thus lead to a reduction in the dose required.

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