Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 651–657
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Magnesium cation effect on passive diffusion of statin molecules: Molecular chromatography approach

Fatimata Seydou Sarr, Yves Claude Guillaume *, Claire André

Equipe des Sciences Séparatives et Biopharmaceutiques (2SB/EA-3924), Laboratoire de Chimie Analytique, Faculté de Médecine Pharmacie, Université de Franche-Comté, Place Saint Jacques, 25030 Besançon Cedex, France

Received 12 November 2007; received in revised form 31 January 2008; accepted 6 February 2008
Available online 16 February 2008

Abstract

Recently, immobilized artificial membranes (IAMs) have been introduced as HPLC column packing materials. IAMs consist of phosphatidylcholine residues, the most common phospholipids in natural membranes, covalently bound to silica propylamine and consequently mimic fluid phospholipid bilayer. Thus, the immobilized artificial membrane provided a biophysical model system to study the passive diffusion of the statin molecules through the cellular membrane. Statins or 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA R) inhibitors are widely used for reducing the circulating atherogenic lipid fractions and decreasing cardiovascular morbidity and mortality. This paper describes magnesium cation (Mg^{2+}) effect on five statin molecules (pravastatin, mevastatin, atorvastatin, simvastatin and fluvastatin)–IAM surface association using a molecular chromatography approach. An analysis of the thermodynamics (i.e. enthalpy (ΔH°), entropy (ΔS°*)) of the interaction of the statin molecules with the immobilized monolayer was also carried out. The ΔH° and ΔS°* values were negative due to van der Waals interactions and hydrogen bonding between the statin molecules with the polar head groups of phospholipid monolayer (polar retention effect). However, the increase of statin–IAM association, with the Mg^{2+} concentration increase, was associated with an increase of these thermodynamic data. This explains that this interaction was also governed by hydrophobic and electrostatic bonds which became preponderant. The statin elution order was: Pravastatin $\lll <$ Mevastatin $\ll$ Atorvastatin $\lll$ Simvastatin $<$ Fluvastatin. This result confirmed that pravastatin, which exhibited the lowest association with the lipid monolayer, was taken up by a membrane transporter. It appeared equally that Mg^{2+} supplementation (Mg^{2+} concentration range 0.0–2.6 mmol L^{-1}, including its biological concentration range, i.e. 0.75–0.90 mmol L^{-1}) could increase the statin passive diffusion into hepatocytes and their pharmacological actions on cholesterol biosynthesis.
© 2008 Elsevier B.V. All rights reserved.

Keywords: Statins; Binding; IAM; Magnesium cation

1. Introduction

High plasma cholesterol has been acknowledged, since mid-20th century, as a major heart disease risk factor [1–3]. Indeed, hypercholesterolemia plays a crucial role in the development of atherosclerotic diseases in general and coronary heart disease in particular [2–4]. Several studies showed that serum magnesium concentration has been reported to be inversely associated with atherogenic lipid fractions. Indeed, the magnesium deficit is an important factor in the physiology of the cardiovascular apparatus and the pathogenesis of cardiovascular diseases [5–7]. More recently, introduced drugs such as statins and fibrates are widely used for reducing the circulating atherogenic lipid fractions and decreasing cardiovascular morbidity and mortality [8]. Statins or 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors are the first-line pharmacologic therapy for hypercholesterolemia [9,10]. Statins drugs represent the most successful class of drugs for the treatment of hypercholesterolemia and dyslipidaemia implicated in the pathogenesis of coronary heart disease and atherosclerosis [11,12]. Atherosclerotic disease is considered to be the leading cause of death and loss of disability-adjusted life-years worldwide [13]. Statins, which are reversible inhibitors of microsomal enzyme HMG-CoA reductase, play an important part in the reduction of plasma cholesterol levels more precisely low density lipoprotein (LDL) cholesterol [14]. Studies have demonstrated that statin adverse effects (like myositis and rhabdomyolysis (severe myopathy involving muscle breakdown) and

* Corresponding author. Tel.: +33 3 81 66 55 44; fax: +33 3 81 66 56 55.
E-mail address: yves.guillaume@univ-fcomte.fr (Y.C. Guillaume).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.02.008

pharmacokinetic interactions are linked to the increase of this systemic bioavailability [15,16]. Recent work showed that the beneficial effects of the statin drugs are paralleled and complemented by those of magnesium [1]. Indeed, Mg^{2+}-ATP is the controlling factor for the rate-limiting enzyme in the cholesterol biosynthesis sequence that is targeted by the statin pharmaceutical drugs. Statins, like Mg, have activities important in cardiovascular and overall health [1]. Indeed, statins and magnesium inactivate the enzyme, HMG-CoA reductase, which converts HMG-CoA to mevalonate, the initial step in cholesterol biosynthesis [1].

Statins, drugs of the 21st century, were used by 20 million people and are the most prescribed medications in the world [17]. Therefore, it is very important to know pharmacologic and pharmacokinetic processes of their lipid-lowering drugs in human because the ability to cross biological membrane strongly affects the pharmacokinetic behaviour of drugs and their capacity to access the receptor site. At present, the reference parameter to predict passive diffusion through the biological barriers is lipophilicity expressed as the logarithm of the partition coefficient between on organic solvent and on aqueous phase ($\log P$) [18]. This parameter is however only useful when polar group interactions between the solute and the phospholipids bilayers are minimal or absent [15]. It lacks structural similarities to cell membranes, reflecting only the hydrophobicity of a compound and is not suitable for highly polar and ionic compounds [19,20]. Since phospholipids are the main lipidic constituents of biomembranes, their use as partitioning phase can be expected valuable to mimic drug–biomembrane interactions.

Recently, immobilized artificial membranes (IAMs) have been introduced as HPLC column packing materials and this development unfolded new perspectives for rapid evaluation of drug partitioning into cell membranes [16,21]. IAMs consist of phosphatidylcholine residues covalently bound to silica propylamine and consequently mimic fluid phospholipid bilayer [22,23]. This technique is an acceptable method for the prediction of membrane permeability of drugs [16,21,24] particularly for ionisable compound due to the fact that the position of the polar compounds in biomembranes is strongly affected by electrostatic and/or hydrogen bound interactions with phospholipids [25,26]. Excellent correlations have been demonstrated between IAM chromatography indices and biological systems such as the prediction of the intestinal absorption of structurally diverse compounds [21,24,27] and of skin permeability coefficients [28]. IAM technique provided a biophysical model system to study the passive diffusion of the statin molecules through the cell membrane.

As the beneficial effects of the statin drugs are paralleled and complemented by those of magnesium and as pharmacokinetic interactions are reflected by the statin bioavailability, the aim of this study was to investigate the effect of the Mg^{2+} concentrations in the bulk solvent (i.e. the mobile phase) and the column temperature T on the binding process of five statin molecules with IAM. The shapes of the van't Hoff plots were used to assess the effects of temperature and Mg^{2+} concentrations in the mobile phase on the statin–IAM association. The thermodynamic data corresponding to the statin–IAM physico-chemical process were calculated. As well, enthalpy–entropy compensation of this association was investigated to evaluate the main parameter controlling this binding mechanism.

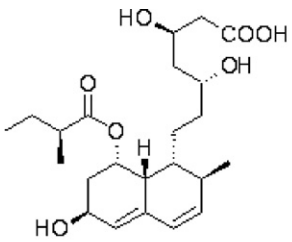
2. Experimental and method

2.1. Apparatus

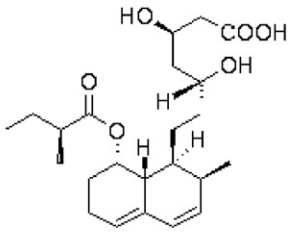
The high performance liquid chromatography (HPLC) system was consisted of a Hitachi L 7100 pump (Merck, Nogent sur Marne, France), a Rheodyne 7125 injection valve (Cotati, California, USA) fitted with a 20 μL sample loop, and a Shimadzu UV–Visible detector. Retention data of statins were obtained with a 100.0 mm × 4.6 mm IAM-PC-DD2 (immobilized artificial membrane-phosphatidylcholine-drug discovery) packing (Interchim, Montluçon, France). The stationary phase of this column was consisted of diacyl double chain ester phosphatidylcholine (PC) ligands surface-bounded to an aminopropylsiloxane-bonded silica substrate and was end capped by mixed propionic and decanoic alkylamine groups. A major advantage of this particular IAM chromatographic stationary phase (i.e. IAM-PC-DD) is that it had the shortest retention times of commercialized IAM packing [29–31]. It was also more readily and reproducibility synthesized commercially. Moreover, the IAM-PC-DD packing was stable like the ester packing leading a better modelization of the drug membrane transport [30,31]. Data were calculated using the Microsoft office Excel 2003 software.

2.2. Solvent and samples

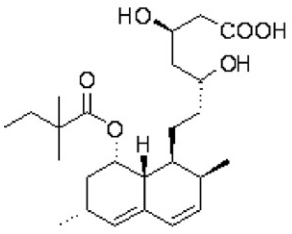
The five statins (STCOOH) were depicted in Fig. 1. Pravastatin (prava), mevastatin (meva), atorvastatin (atorva), simvastatin (simva) and fluvastatin (fluva) were purchased from Sigma and VWR (Paris, France). Water was obtained from an Elgastat option water purification (Odil Talant, France) fitted with a reverse osmosis cartridge. Methanol was supplied by Carlo Erba (Val de Reuil, France). Boric acid (0.2 mmol L^{-1}) and tetraborate (0.05 mmol L^{-1}) were obtained from Prolabo and Merck (Paris, France), respectively. The mobile phase was consisted of 60/40 (v/v) tetraborate buffer–methanol with magnesium (Mg^{2+}) concentrations equal to 0.0, 0.6, 1.0, 1.4, 1.8, 2.0, 2.4 and 2.6 mmol L^{-1} (including its biological concentration range, i.e. 0.75–1.0 mmol L^{-1}). Each mobile phase was adjusted to a pH value equal to7.0. The buffer was stocked for 1, 2 and 4 h at ambient room temperature to study the accuracy of their pH values. No fluctuations were observed. The maximum relative difference of mobile phase pH value was always lower than 0.4% [32]. Experiments were carried out over the temperature range 5–45 °C (5, 10, 15, 20, 25, 30, 35, 40 and 45 °C) and at a 254 nm detection wavelength. The chromatographic system was left to equilibrate at each temperature for at least 30 min before each experiment. To study this equilibration, the retention time of fluvastatin was measured after 22, 23 and 24 h. The maximum relative difference between retention times of this solute molecule was never more than 0.7%, meaning that after 30 min the chromatographic system was sufficiently equi-



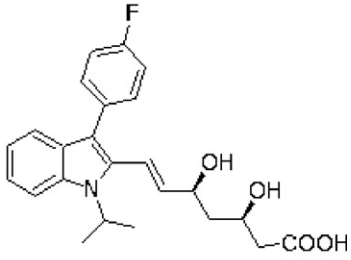
Pravastatin



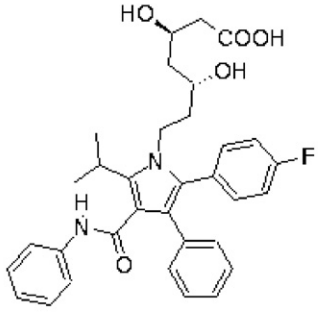
Mevastatin



Simvastatin



Fluvastatin



Atorvastatin

Fig. 1. Chemical structures of the statins (STCOOH).

librated for use [33]. Throughout the study, the flow rate was maintained constant and equal to 0.5 mL/min.

2.3. Thermodynamic relationships

The affinity chromatography IAM stationary phase allows to study the statin interaction on the IAM surface. Indeed, the retention factor (k') is proportional to the association constant K of the statin on the IAM surface and can be described by the following equation:

$$k' = \Phi K \tag{1}$$

where Φ is the IAM column phase ratio (volume of the stationary phase divided by the volume of the mobile phase).

The solute binding to the IAM can be expressed in terms of retention factor k' using the well-known van't Hoff equation:

$$\ln k' = \frac{-\Delta H^\circ}{RT} + \Delta S^{\circ *} \tag{2}$$

$$\Delta S^{\circ *} = \frac{\Delta S^\circ}{R} + \ln \Phi \tag{3}$$

where R is the gas constant (8.32 J/mol/K), T is the column temperature in Kelvin, ΔH° and ΔS° are, respectively, the solute enthalpy and entropy changes accompanying the transfer of the statins from the bulk solvent to the IAM surface. ΔH° and $\Delta S^{\circ *}$ can be calculated from the slope and intercept of linear van't Hoff plot. This provides a suitable way to estimate the thermodynamic constants ΔH° and ΔS° if the phase ratio is known or can be calculated. Although, ΔS° is not usually provided because of the

ambiguity in the phase ratio for commercial use, $\Delta S^{\circ}*$ varies identically with ΔS°.

2.4. Bulk solvent Mg^{2+} effects

When the Mg^{2+} of the bulk solvent changed, a fuller description is essential, which explicitly maintains conservation of mass of each species and take into account binding of Mg^{2+} to the IAM, STCOOH, and the complex IAM·STCOOH:

$$\mathrm{IAM}(\mathrm{Mg}^{2+})_A + \mathrm{STCOOH}(\mathrm{Mg}^{2+})_B + n_{\mathrm{Mg}^{2+}}\mathrm{Mg}^{2+} \rightleftarrows \mathrm{IAM}\cdot\mathrm{STCOOH}(\mathrm{Mg}^{2+})_C \quad (4)$$

where $n_{\mathrm{Mg}^{2+}} = C - (A + B)$ is the number of Mg^{2+} linked to this STCOOH binding reaction of IAM.

The association constant of this equilibrium was given by:

$$K = \frac{[\mathrm{IAM}\cdot\mathrm{STCOOH}]}{[\mathrm{IAM}]\,[\mathrm{STCOOH}]\,[\mathrm{Mg}^{2+}]^{n_{\mathrm{Mg}^{2+}}}} \quad (5)$$

Eq. (5) can be rewritten as:

$$K = \frac{K_0}{[\mathrm{Mg}^{2+}]^{n_{\mathrm{Mg}^{2+}}}} \quad (6)$$

where K_0 is the K values for $n_{\mathrm{Mg}^{2+}} = 0$. Taking the logarithm of Eq. (6) gives:

$$\log K = \log K_0 - n_{\mathrm{Mg}^{2+}} \log[\mathrm{Mg}^{2+}] \text{ with } -\log[\mathrm{Mg}^{2+}] = P\mathrm{Mg}^{2+} \quad (7)$$

After derivation of Eq. (7) we obtained:

$$\frac{\partial \log K}{\partial P\mathrm{Mg}^{2+}} = n_{\mathrm{Mg}^{2+}} \quad (8)$$

Combining (1) and (8), the following is obtained:

$$\frac{\partial \log k'}{\partial P\mathrm{Mg}^{2+}} = n_{\mathrm{Mg}^{2+}} \quad (9)$$

3. Results and discussion

The retention factor k' of each statin under study was determined with the immobilized artificial membrane in the entire range of temperature, i.e. from 5 to 35 °C. Experiments were carried out at pH 7.0 and with various Mg^{2+} concentrations (0.0, 0.6, 1.0, 1.4, 1.8, 2.0, 2.4, 2.6 and 3.0 mmol L^{-1}) in the buffer solvent. Eq. (2) showed that with an invariant statin-biomembrane association mechanism over the temperature range being studied, the association enthalpy ΔH° remained constant and a plot of $\ln k'$ in relation to $1/T$ leads to a straight line with an enthalpic slope and entropic origin. For all statins, when the temperature increased there was a decrease in the statin-biomembrane association. Linear van't Hoff plots were obtained with correlation coefficients r higher than 0.96 for all fits for all the magnesium concentrations studied. This linear behaviour is thermodynamically expected if the statin-biomembrane association mechanism is independent of temperature. According to Eq. (2) the thermodynamic parameters (ΔH° and $\Delta S^{\circ}*$) were calculated. All the

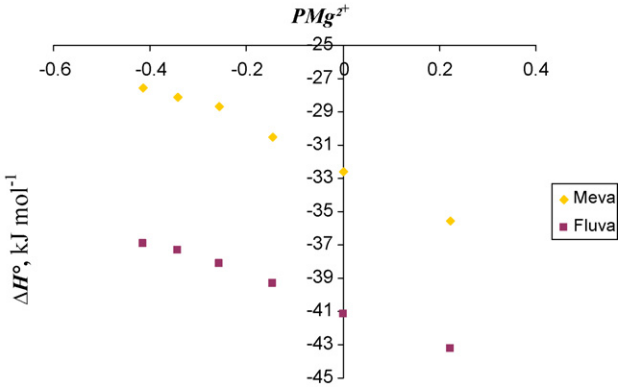


Fig. 2. Enthalpies (ΔH°, kJ mol^{-1}) of meva and fluva vs. $P\mathrm{Mg}^{2+}$.

statins exhibited a similar linear variation ($r^2 > 0.85$) for the thermodynamic data regardless of the $P\mathrm{Mg}^{2+}$ (Figs. 2 and 3). For example, the corresponding equations for fluvastatin were:

$$\Delta H^{\circ} = -10.33\,P\mathrm{Mg}^{2+} - 40.96, \quad r = 0.998 \quad (10)$$

$$\Delta S^{\circ}* = -5.30\,P\mathrm{Mg}^{2+} - 14.42, \quad r = 0.998 \quad (11)$$

Negative enthalpies indicated that it was energetically more favourable for the statin molecule to be associated with the lipid surface. Negative entropies showed an increase in the order of the chromatographic system when the statin molecules were transferred from the bulk solvent to the phospholipid monolayer (IAM). The negative value of the thermodynamic data was usually the case for several pharmacomolecule-IAM association [34]. This transfer was enthalpically driven and can be described as the replacement of weak statin/solvent interactions by strong statin/lipid surface interactions. This indicates that enthalpic factors associated with hydrogen bonding and van der Waals interactions (characterized by negative enthalpy changes at these temperatures) [35] of the statin molecules with the lipid monolayer dominate the binding rather than entropic factors relate to the changes in the mobility of the statin molecules and the lipid monolayer fluidity. These results confirmed the great importance of the polar sites of the IAM surface on this association process (polar retention effect [34]). On the IAM surface, the elution order of statin molecules at all the Mg^{2+}

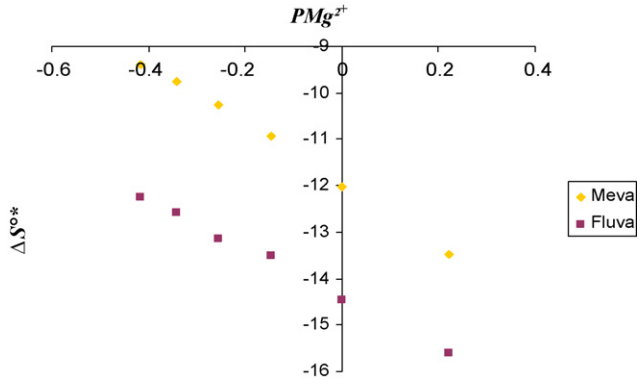


Fig. 3. Entropies ($\Delta S^{\circ}*$, no units) of meva and fluva vs. $P\mathrm{Mg}^{2+}$.

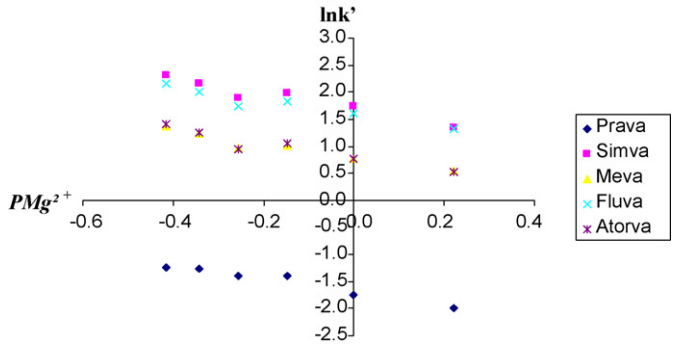


Fig. 4. $\ln k'$ for all statins vs. $P\mathrm{Mg}^{2+}$ ($-\log \mathrm{Mg}^{2+}$) in the bulk solvent at a column temperature equal to 35 °C.

concentrations remained constant:

Prava ≪≪ Meva ≪ Atorva ≪< Simva < Fluva

Pravastatin exhibited the lowest association with the lipid monolayer. This can be explained by the lowest hydrophobicity of this statin molecule [14,36]. IAM, which is only constituted by a phospholipid monolayer, allowed to study the statin passive diffusion [21]. Thus, this result confirmed the studies which showed that pravastatin was taken by a membrane transporter [37–39]. Among the five statins, fluvastatin exhibited the lowest thermodynamic data and the highest affinity with the IAM surface. For example, at $[\mathrm{Mg}^{2+}] = 1.00\,\mathrm{mmol\,L^{-1}}$ $\Delta H^\circ = -41.13\,\mathrm{kJ\,mol^{-1}}$ and $\Delta S^\circ* = -14.46$ (no units). This can be explained by the hydrophobicity of this statin molecule [36]. As well the polar substituent on the compound (–F substituent, two –OH substituents) on the fluvastatin enhanced the lipid surface affinity by increasing hydrogen bonds between the solute molecule and the polar head groups of the IAM surface. This was associated with the lowest entropy state classically attributed to the release of the water molecules surrounding the solute molecule when the fluvastatin was transferred into the phospholipid monolayer [40].

In order to elucidate the effect of Mg^{2+} cation concentrations in the bulk solvent on the statin–IAM association, the logarithm of the retention factor k' were plotted against $P\mathrm{Mg}^{2+}$ for each statin molecule and for a large variation range of $P\mathrm{Mg}^{2+}$ ($-0.42 \leq P\mathrm{Mg}^{2+} \leq 0.22$). The slope of the plots $\ln k'$ vs. $P\mathrm{Mg}^{2+}$ was similar for all statins. Fig. 4 reported the curves obtained at $T = 35\,^\circ\mathrm{C}$ for all the statins. These plots showed that the statin retention was Mg^{2+} dependent. Indeed, statin–IAM association increased significantly with salt concentration increase in the bulk solvent. Then, in the biological Mg^{2+} concentration (0.75–0.90 mmol $\mathrm{L^{-1}}$), an increase of Mg^{2+} led an enhancement of the statin–IAM association (i.e. an increase of the statin passive diffusion). It has been known for several years that increasing the ionic strength of a bulk solvent increased its surface tension [41–43]. Increasing statin–IAM association can be classically attributed to the osmotropic character of the magnesium cation [41]. In fact, increasing Mg^{2+} concentration in the bulk solvent involved a decrease of the water solubility of apolar compounds by electrostriction of the bulk solvent (i.e. increase in bulk mobile phase surface tension) [42]. Previous studies had demonstrated that this change in water activity (i.e. the hydrophobic effect) played an important role in the solute molecule-biomembrane association mechanism [44]. Thus, this change in the water activity led to a favourable statin–IAM binding. The Mg^{2+} cation and the carboxylate group negatively charged of two statin molecules could form an ionic association which could increase the statin hydrophobicity and therefore the statin–IAM association. As well, the Mg^{2+} cation, the carboxylate group of the statin molecule and the phosphate group of the IAM surface could formed a second ionic association [45] which could decrease the electrostatic repulsions between the statin molecule and the IAM surface and therefore lead to a favourable statin–IAM association. Indeed, in a previous paper [46] where the statin–IAM binding mechanism was analyzed at a pH range 5.7–7.0, it was shown that the decrease of this statin–IAM association observed when the pH increased was due to the electrostatic repulsions between the statin carboxylate group negatively charged and the phosphate group of the IAM surface. Consequently, this ion pair formation induced a decrease of the ionic interaction (i.e. the electrostatic repulsions) and then the statin–IAM association was increased. It was also important to note that this favourable statin–IAM association observed when the Mg^{2+} concentration increased was accompanied by an increase of the thermodynamic data corresponding to this molecular association which became progressively less negative. For example, the plots of the thermodynamic terms ΔH° and $\Delta S^\circ*$ of mevastatin and fluvastatin vs. $P\mathrm{Mg}^{2+}$ were illustrated in Figs. 2 and 3. The magnesium cation tends to enhance retention of the statins on the IAM by minimizing the statin contact area exposed to the bulk solvent. Consequently both the statin molar enthalpy and entropy associated to the bulk solvent decreased leading an increase of the thermodynamic data [47] classically attributed to favourable hydrophobic interactions [33,42,48].

From Eq. (9) ($n_{\mathrm{Mg}^{2+}} = \partial \log k'/\partial P\mathrm{Mg}^{2+}$) the slope of the curve $\ln k'$ vs. $P\mathrm{Mg}^{2+}$ (Fig. 4) gives the number of magnesium at the statin–IAM interface implied in the binding process. These values were shown in Table 1. For example, at 35 °C the corresponding $n_{\mathrm{Mg}^{2+}}$ value of fluvastatin was −0.52. The negative values of $n_{\mathrm{Mg}^{2+}}$ reflected the exclusion of Mg^{2+} when statin bound to the phospholipid monolayer. The ionic association between Mg^{2+} and (i) the carboxylate groups of two statin molecules and (ii) the phosphate group of IAM explained

Table 1
The $n_{\mathrm{Mg}^{2+}}$ values of statin–IAM association at all the temperature studied

Temperature (°C)	Prava	Meva	Atorva	Simva	Fluva
5	−0.26	−0.33	−0.34	−0.38	−0.37
10	−0.22	−0.44	−0.41	−0.40	−0.38
15	−0.29	−0.52	−0.50	−0.42	−0.43
20	−0.31	−0.49	−0.52	−0.47	−0.45
25	−0.33	−0.50	−0.53	−0.52	−0.51
30	−0.56	−0.53	−0.53	−0.58	−0.54
35	−0.53	−0.54	−0.56	−0.61	−0.52
40	−0.90	−0.53	−0.62	−0.76	−0.57
45	−0.82	−0.74	−0.76	−0.74	−0.62

the negative number of Mg^{2+} exchanged. These $n_{Mg^{2+}}$ values were temperature dependent. Hinz et al. [49] established that the magnesium binding heat effect ($\Delta H_{Mg^{2+}}$) associated with a magnesium release during a binding reaction can be described by the following simple function:

$$\Delta H_{Mg^{2+}} = -2.3RT^2 \left(\frac{\partial n_{Mg^{2+}}}{\partial T} \right)_{PMg^{2+}} \quad (12)$$

For example, the plot $n_{Mg^{2+}}$ of simvastatin vs. temperature was given in Fig. 5. All the other statins exhibited a similar linear variation ($r^2 > 0.80$). As the temperature increased, the number of magnesium released $n_{Mg^{2+}}$ increased linearly and $(\partial n_{Mg^{2+}}/\partial T)_{PMg^{2+}}$ had a negative value. Thus, from Eq. (12), $\Delta H_{Mg^{2+}}$ has a positive value, and as the Mg^{2+} increased, the binding enthalpy contributes non-favourably to the free energy of binding. As well, using the above relation, the $\Delta H_{Mg^{2+}}$ value was determined at 35 °C for simvastatin and atorvastatin 18.13 and 14.51 kJ mol^{-1}, respectively. These values were in the same order of magnitude as the $\Delta H_{Mg^{2+}}$ values obtained during the Mg^{2+}-human serum albumin (HSA) binding [50].

In order to gain further insight into this binding mechanism, enthalpy–entropy compensation (EEC) was also investigated. Enthalpy–entropy compensation temperature is a useful thermodynamic approach to the analysis of physico-chemical data [44]. Mathematically the entropy–enthalpy compensation can be expressed by the following equation:

$$\Delta H^\circ = \beta \Delta S^\circ + \Delta G^\circ_\beta \quad (13)$$

ΔG°_β is the corresponding Gibbs free energy variation at the compensation temperature β. According to this last equation, when enthalpy–entropy compensation is observed with a group of compounds in a particular chemical interaction, all the compounds have the same free energy ΔG°_β at the temperature β [51,52]. The plots ΔH° vs. $\Delta S^\circ*$ were drawn for all the statin molecules and at all the Mg^{2+} concentrations in the bulk solvent (Fig. 6). The coefficient correlation for the linear fit was equal to 0.83. According to several authors [53,54], it can be deduced that the fraction of the free energy that arises from the enthalpy contributions is the same for all the statin molecules and the Mg^{2+} concentrations in the bulk solvent. Similarly, the fraction of the total free energy arising from the entropy contributions is the same. But, since different mechanisms could result in the same proportion of enthalpy and entropy relative to the overall free energy, it cannot be deduced rigorously that the statin molecule association mechanism on the IAM surface was independent of the statin molecule structure and the Mg^{2+} concentration in the bulk solvent. However, all the studied molecules have similar biological activity on cholesterol biosynthesis [36]. These two conditions (EEC and similar biological effects) seem to imply a similarity of properties of all the statins studied.

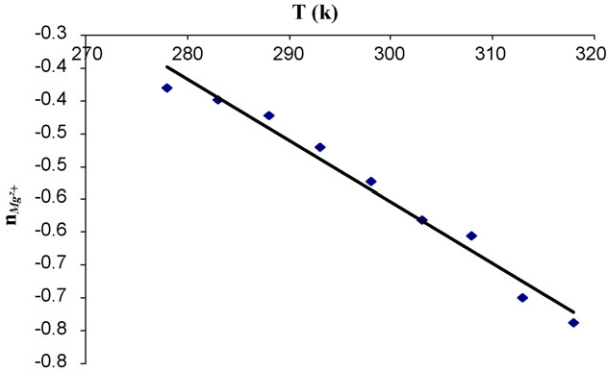


Fig. 5. Temperature dependence of the released magnesium ($n_{Mg^{2+}}$) of simva.

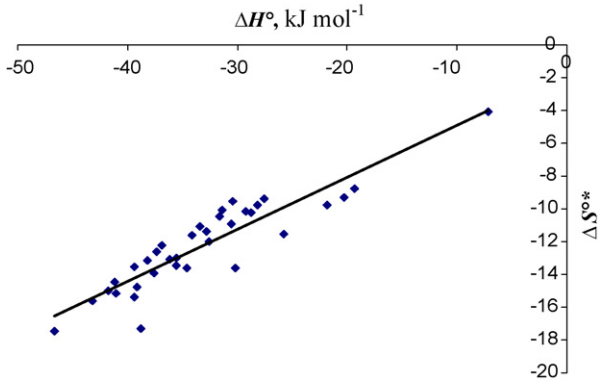


Fig. 6. Enthalpy–entropy compensation of statins at all the Mg^{2+} concentrations.

4. Conclusion

In this paper, the role of the magnesium cation (Mg^{2+}) on the statin–IAM binding mechanism was examined at pH 7.0. It was shown that the statin–IAM association was Mg^{2+} dependent. Indeed, an increase of the Mg^{2+} concentration led an increase of the statin–IAM association classically attributed to salt effect (i.e. hydrophobic bonds) and to ion pair formations between the Mg^{2+} cation and statin molecule to bind to IAM surface. Moreover, the enhancement of the hydrophobic interactions induced an increase of the thermodynamic values which became progressively less negative. These results demonstrated that the mechanism of statin–IAM binding was mainly controlled by hydrogen and van der Waals interactions coupled with hydrophobic and electrostatic bonds. It can be also noted in this study that pravastatin exhibited the lowest association with the IAM lipid monolayer which allowed to study the statin passive diffusion. This result confirmed that the pravastatin uptake into hepatocytes needed a membrane transporter. Enthalpy–entropy compensation suggested that statins have an identical retention mechanism with the phospholipid monolayer. Ultimately, it appeared in this manuscript that, in the biological Mg^{2+} concentration (i.e. 0.75–0.90 mmol L^{-1}), an increase of the Mg^{2+} concentration led an enhancement of the statin–IAM association (i.e. their passive diffusion) and consequently a possible decrease of their systemic bioavailable. Then, this study tends to show that an Mg^{2+} supplementation could increase the statin pharmacological action on cholesterol biosynthesis and probably decrease their adverse effects like myositis and rhabdomyolysis. These results raised important questions that should be answered to further understand the magnesium supplementation effect on the statin passive diffusion through the cellular membrane.

Acknowledgements

This work was supported by Grants 2006 from Direction Générale de la Recherche from Programme National de Biotechnologie, Ministère de la Recherche, France.

References

[1] A. Rosanoff, M.S. Seelig, J. Am. Coll. Nutr. 23 (2004) 501–505.
[2] G. Devuyst, J. Bogousslavsky, Schweiz. Med. Wochenschr. 130 (2000) 1157–1163.
[3] T. Carbonell, E. Freire, Biochemistry 44 (2005) 11741–11748.
[4] H. Lennernäs, G. Fager, Clin. Pharmacokinet. 32 (1997) 403–425.
[5] A. Haenni, M. Öhrvall, H. Lithell, Metabolism 50 (2001) 1147–1151.
[6] Y.C. Guillaume, L. Nicod, T.T. Truong, C. Guinchard, J.F. Robert, M. Thomassin, J. Chromatogr. B 768 (2002) 129–135.
[7] Y. Rayssiguier, E. Gueux, L. Bussière, J. Durlach, A. Mazur, J. Am. Coll. Nutr. 12 (1993) 133–137.
[8] S.X. Kong, S.Y. Crawford, S.K. Gandhi, J.D. Seeger, G.T. Schumock, N.P. Lam, J. Stubbings, M.D. Schoen, Clin. Ther. 19 (1997) 778–797.
[9] D.J. Maron, S. Fazio, M.F. Linton, Circulation 101 (2000) 207–213.
[10] Y. Shitara, M. Hirano, H. Sato, Y. Sugiyama, J. Pharmacol. Exp. Ther. 311 (2004) 228–236.
[11] A. Tiwari, V. Bansal, A. Chugh, K. Mookhtiar, Expert Opin. Drug Saf. 5 (2006) 651–666.
[12] S. Bellosta, R. Paoletti, A. Corsini, Circulation 109 (2004) 50–57.
[13] D. Vinereanu, Herz 3 (2006) 5–24.
[14] C. Chen, R.J. Mireles, S.D. Campbell, J. Lin, J.B. Mills, J.J. Xu, T.A. Smolarek, Drug Metab. Dispos. 33 (2005) 537–546.
[15] J.A. Rogers, A. Wong, Int. J. Pharm. 6 (1980) 339–348.
[16] S. Ong, H. Lui, C. Pidgeon, J. Chromatogr. A 774 (1996) 113–128.
[17] M.H. Davidson, P.P. Toth, Prog. Cardiovasc. Dis. 47 (2004) 73–104.
[18] A.J. Leo, C. Hanoch, D. Elkins, Chem. Rev. 71 (1971) 525–615.
[19] L.G. Herbette, G. Gaviraghi, T. Tulenko, R.P. Mason, J. Hypertens. Suppl. 11 (1993) 13–19.
[20] S. Ong, H. Liu, X. Qui, G. Bhat, C. Pidgeon, Anal. Chem. 67 (1995) 755–762.
[21] C. Pidgeon, S. Ong, H. Lui, M. Pidgeon, A.H. Dantzig, J. Munroe, W.J. Hornback, J.S. Kasher, J.S. Glunz, T. Szczerba, J. Med. Chem. 38 (1995) 590–594.
[22] J. Li, J. Sun, S. Cui, Z. He, J. Chromatogr. A 1132 (2006) 174–182.
[23] T.E. Yen, S. Agatonovic-Kustrin, A.M. Evans, R.L. Nation, J. Ryand, J. Pharm. Biomed. Anal. 38 (2005) 472–478.
[24] M. Genty, G. Gonzàlez, C. Clere, V. Desangle-Gouty, J.Y. Legendre, Eur. J. Pharm. Sci. 12 (2001) 223–229.
[25] A. Avdeef, K.S. Box, J.E.A. Comer, C. Hibbert, K.Y. Tom, Pharm. Res. 15 (1998) 209–215.
[26] F. Barbato, G. Di Martino, L. Grumetto, M.I. La Rotondo, Pharm. Sci. 22 (2004) 261–269.
[27] E. Deconinck, H. Ates, N. Callebaut, E. van Gyseghem, Y. vander Heyden, J. Chromatogr. A 1138 (2007) 190–202.
[28] A. Nasal, M. Sznitowska, A. Bucinski, R. Kaliszan, J. Chromatogr. A 692 (1995) 83–89.
[29] G.W. Caldwell, J.A. Masucci, M. Evangelisto, R. White, J. Chromatogr. A 800 (1998) 161–169.
[30] S. Ong, S.J. Cai, C. Bernal, D. Rhee, X. Qui, C. Pidgeon, Anal. Chem. 66 (1994) 782–792.
[31] C. Pidgeon, U.V. Venkataran, Anal. Biochem. 176 (1989) 36–47.
[32] C. Andre, Y.C. Guillaume, Talanta 63 (2004) 503–508.
[33] C. Andre, L. Ismaili, J. Millet, M. Thomassin, Y.C. Guillaume, Chromatographia 57 (2003) 771–776.
[34] C. Andre, L. Ping, M. Thomassin, J.F. Robert, Y.C. Guillaume, Anal. Chim. Acta 542 (2005) 199–206.
[35] P.D. Ross, S. Subramanian, Biochemistry 20 (1981) 3096–3102.
[36] B.A. Hamelin, J. Turgeon, Trends Pharmacol. Sci. 19 (1998) 26–37.
[37] J.W. Park, R. Siekmeier, M. Merz, B. Krell, S. Harder, W. März, D. Seidel, S. Schüler, W. Gross, Int. J. Clin. Pharmacol. Ther. 40 (2002) 439–450.
[38] B. Hsiang, Y. Zhu, Z. Wang, Y. Wu, V. Sasseville, W.P. Yang, T.G. Kirchgessner, J. Biol. Chem. 274 (1999) 37161–37168.
[39] M. Yamazaki, H. Suzuki, M. Hanano, T. Tokui, T. Komai, Y. Sugiyama, Am. J. Physiol. 264 (1993) 36–44.
[40] C. Andre, C. Guyon, M. Thomassin, A. Barbier, L. Richert, Y.C. Guillaume, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 820 (2005) 9–14.
[41] M. Yanado, Y. Yano, M. Umara, Y. Kondo, J. Solut. Chem. 24 (1995) 587–592.
[42] C.J. Van Oss, Interfacial Forces in Aqueous Media, Marcel Dekker, New York, 1994.
[43] C. Tanford, The Hydrophobic Effect, Wiley/Interscience, New York, 1973.
[44] W. Melander, C.S. Horvath, High Performance Chromatography; Advances and Perspectives, vol. 23, Academic Press, New York, 1986.
[45] L. Ismaili, B. Refouvelet, A. Xicluna, J.F. Robert, Y.C. Guillaume, J. Pharm. Biomed. Anal. 32 (2003) 549–553.
[46] F.S. Sarr, C. Andre, Y.C. Guillaume, J. Chromatogr. B, submitted for publication.
[47] C. Andre, A. Berthelot, J.F. Robert, M. Thomassin, Y.C. Guillaume, J. Pharm. Biomed. Anal. 33 (2003) 911–921.
[48] Y.C. Guillaume, T.T. Truong, J. Millet, L. Nicod, J.C. Rouland, M. Thomassin, J. Chromatogr. A 955 (2002) 197–205.
[49] H.J. Hinz, D.D. Shiao, J.M. Sturtevant, Biochemistry 10 (1971) 1347–1352.
[50] Y.C. Guillaume, C. Guinchard, A. Berthelot, Talanta 53 (2000) 561–569.
[51] R.R. Krug, Ind. Eng. Chem. Fundam. 19 (1980) 50–59.
[52] Y. Matsui, K. Mochida, Bull. Chem. Soc. Jpn. 52 (1979) 2808–2814.
[53] J. Li, P. Carr, J. Chromatogr. 12 (1994) 105–116.
[54] R. Ranatunga, M.F. Vitha, P. Carr, J. Chromatogr. A 946 (2002) 47–49.