Binding Thermodynamics of Statins to HMG-CoA Reductase[†]

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ABSTRACT: The statins are powerful inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase), the key enzyme in the cholesterol biosynthetic pathway, and are among the most widely prescribed drugs in the world. Despite their clinical importance, little is known about the binding thermodynamics of statins to HMG-CoA reductase. In this paper, we report the results of inhibition kinetics and microcalorimetric analysis of a representative type I statin (pravastatin) and four type II statins (fluvastatin, cerivastatin, atorvastatin, and rosuvastatin). Inhibition constants (K_i) range from 2 to 250 nM for the different statins. Isothermal titration calorimetry (ITC) experiments yield binding enthalpies ($\Delta H_{binding}$) ranging between zero and −9.3 kcal/mol at 25 °C. There is a clear correlation between binding affinity and binding enthalpy: the most powerful statins bind with the strongest enthalpies. The proportion by which the binding enthalpy contributes to the binding affinity is not the same for all statins, indicating that the balance among hydrogen bonding, van der Waals, and hydrophobic interactions is not the same for all of them. At 25 °C, the dominant contribution to the binding affinity of fluvastatin, pravastatin, cerivastatin, and atorvastatin is the entropy change. Only for rosuvastatin does the enthalpy change contribute more than 50% of the total binding energy (76%). Since the enthalpic and entropic contributions to binding originate from different types of interactions, the thermodynamic dissection presented here provides a way to identify interactions that are critical for affinity and specificity.

Hypercholesterolemia is the most important risk factor for cardiovascular diseases (CVD) as reported in several epidemiological studies (*1*−*3*). Consequently, the development of powerful and safe drug therapies aimed at lowering cholesterol levels in plasma has been a very important goal. Among the existing cholesterol-lowering drugs, the statins [or 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase)[1] inhibitors] are the most commonly used and the ones that have been shown to reduce the concentration of low-density lipoprotein cholesterol (LDL-C) in serum the most. In fact, several reports have shown that statins significantly reduce mortality and morbidity in patients suffering from CVD (*4*−*13*).

Statins lower cholesterol levels by inhibiting in a competitive manner the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthetic pathway, HMG-CoA reductase. This step involves the conversion of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) into mevalonate, which is the precursor of cholesterol and other isoprenoids (*14*). Recently, several side effects have been associated with the use of statins. While some of those side effects are fortuitously beneficial like anti-inflammatory properties (*15*, *16*) or plaque stabilization (*17*−*20*), others are highly detrimental, like those associated with the fatal cases of rhabdomyolysis that led to the withdrawal of cerivastatin in 2001 (*21*, *22*). Not all statins elicit the same side effects or induce them with the same intensity, suggesting that those differences are associated with their distinct chemical structures. Side effects can originate from different causes, including binding to unwanted targets due to poor selectivity, alteration of downstream metabolic pathways, generation of unsafe catabolic products, etc. At the molecular level, side effects are often triggered by a lack of binding selectivity that results in the binding of the drug molecules to proteins different from its intended target. Nonspecific binding can be related to the type of forces that drive the binding reaction. For example, compounds that rely heavily on nonspecific interactions such as the hydrophobic effect are more likely to exhibit low selectivity than those driven by highly specific interactions. The type and magnitude of forces that drive the binding reaction are reflected in the thermodynamic signature of the drug molecules. Despite their clinical importance, little is known about the thermodynamics of binding of statins to HMG-CoA reductase. As a first step in understanding the binding mechanism of the statins, we have performed an experimental thermodynamic dissection of statin binding to HMG-CoA. It is expected that the availability of structural and thermodynamic binding information should help in the identification of features that might be associated with nonspecific binding.

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[1] Abbreviations: IPTG, isopropyl β-D-thiogalactopyranoside; LB, Luria-Bertani medium; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; $NADP^+$, nicotinamide adenine dinucleotide phosphate, oxidized form; CoASH, coenzyme A, reduced form; Tris-HCl, 2-amino-2-(hydroxymethyl)-1,3-propanediol, hydrochloride; TCEP, tris(carboxyethyl)-phosphine.

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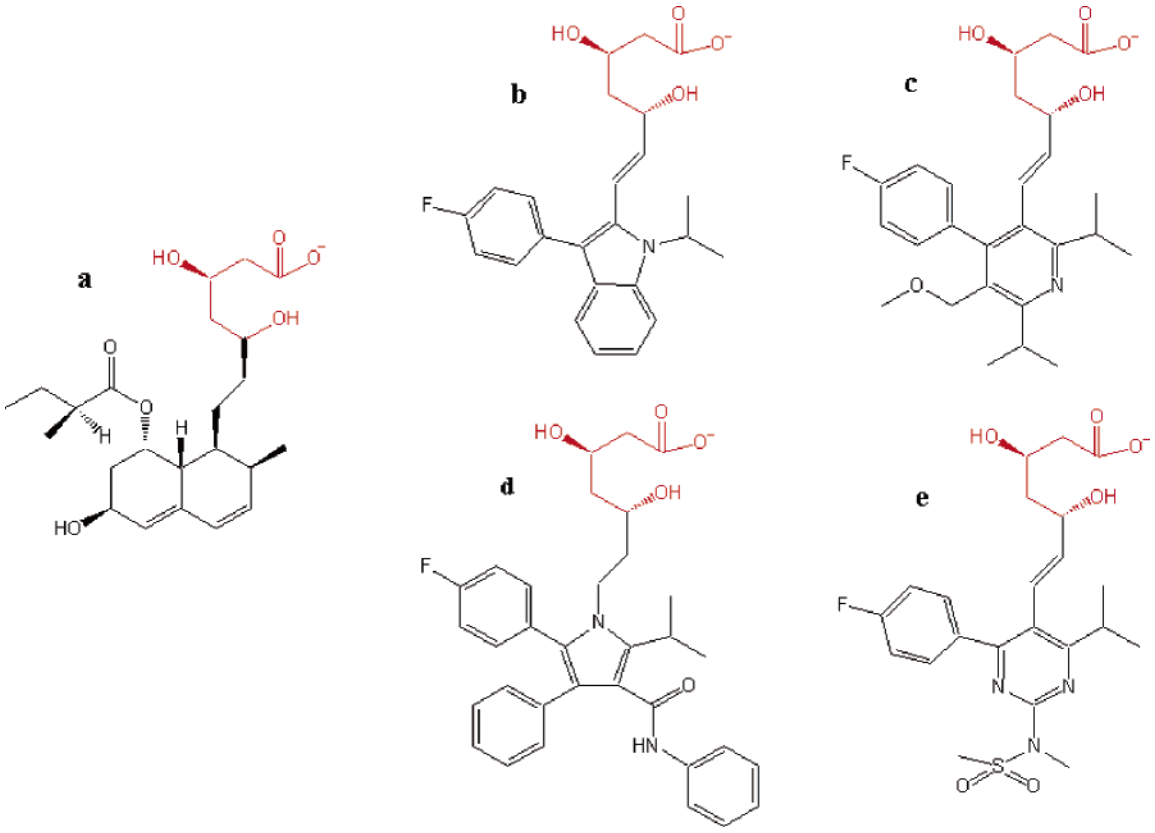

FIGURE 1: Chemical structures of the statins considered in these studies: (a) pravastatin, (b) fluvastatin, (c) cerivastatin, (d) atorvastatin, and (e) rosuvastatin. The HMG moiety common to all statins is colored red and the variable hydrophobic region black.

Although all the statins in clinical use are analogues of the substrate HMG-CoA, they are classified in two groups according to their molecular structures: type I and type II statins (Figure 1) (*23*). From a structural point of view, all statins share the same HMG moiety (shown in red) and differ by the presence of different functionalities attached to the core structure. These functionalities are collectively called the hydrophobic region (shown in black) in the literature (*23*). Type I statins were originally isolated from certain fungi and represent the first generation of these drugs. Lovastatin and simvastatin are administered as lactone pro-drugs that become hydrolyzed by cellular enzymes. On the other hand, type II statins are fully synthetic and are characterized by the presence of hydrophobic regions larger than those in type I statins (*3*, *24*−*27*). In this paper, we report the results of inhibition kinetics and microcalorimetric analysis of a representative type I statin (pravastatin) and four type II statins (fluvastatin, cerivastatin, atorvastatin, and rosuvastatin).

MATERIALS AND METHODS

Protein Expression and Purification. The expression plasmid containing the catalytic subunit corresponding to the human HMG-CoA reductase was kindly provided by J. Deisenhofer (Howard Hughes Institute and Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX). Expression and purification of the catalytic subunit were carried out as described previously (*28*). Briefly, after transformation of *Escherichia coli* BL(DE3) cells, they were grown at 37 °C until they reached an OD_{600} of 0.8. Cells were induced with 1 mM IPTG and grown at 25 °C for 4 h, in LB supplemented with 0.5 M sorbitol and 2.5 mM betaine. Purification of the fusion protein was carried out using a GST affinity column attached to an ÄKTA-FPLC system (Amersham Biosciences). The purity of the protein was checked by SDS−PAGE.

Statin Purification. Fluvastatin, pravastatin, cerivastatin, atorvastatin, and rosuvastatin were purified from commercial pills using a RP-C18 Zorbax column and a reverse-phase high-pressure liquid chromatography (RP-HPLC) system (Agilent Technologies Inc.). Elution of the statins was performed by using a linear gradient of water (0.05% TFA) and acetonitrile (0.05% TFA) that was optimized for each statin. After the molecular weight of the statins had been checked by mass spectrometry, the fractions containing them were collected, lyophilized, and dissolved in 100% DMSO at a final concentration of 20 mM for further experiments.

Enzymatic Assays. Enzymatic assays were performed in 100 mM sodium phosphate buffer (pH 6.8) containing 100 mM NaCl, 1 mM EDTA, 10 mM DTT, 2% DMSO, and 4 nM enzyme in a final volume of 300 μL at 37 °C (*29*). For HMG-CoA K_m determination, 270 μM NADPH was added to the buffer and the substrate concentration (i.e., HMG-CoA) was varied between 0 and 400 μM. The absorbance at 340 nm was monitored, and the reaction rates were taken from the initial slope. The same procedure was utilized for NADPH K_m determination; in this case, the NADPH concentration was varied between 0 and 500 μM and the HMG-CoA concentration was kept constant at 100 μM. For inhibition constant (K_i) determination, 100 μM HMG-CoA and 270 μM NADPH were added to the 100 mM sodium phosphate buffer (pH 6.8), 100 mM NaCl, 1 mM EDTA, 10

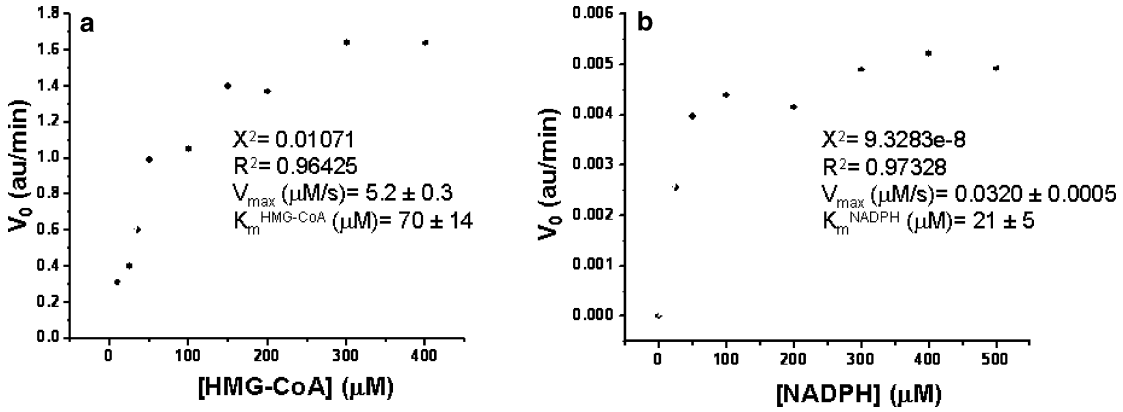

FIGURE 2: K_m determination for HMG-CoA reductase for its substrate HMG-CoA (a) and NADPH (b). Experiments were performed at 37 °C in 100 mM sodium phosphate (pH 6.8), 100 mM NaCl, 1 mM EDTA, 10 mM DTT, and 2% DMSO.

mM DTT, and 2% DMSO. Increasing concentrations of each statin (ranging from 0 to 50 μM) were incubated for 15 min with HMG-CoA reductase (final concentration of 4 nM) before HMG-CoA was added. In each case, the absorbance at 340 nm was monitored immediately after the addition of HMG-CoA. Reaction rates were obtained from the initial slope. Kinetic and inhibition constants were determined by nonlinear least-squares analysis of the data with respect to the Michaelis−Menten kinetic equations as described previously (*30*).

Isothermal Titration Calorimetry Experiments. Isothermal titration calorimetry experiments (ITC), were carried out using a high-precision VP-ITC titration calorimetry instrument (Microcal Inc., Northampton, MA) with each statin and the HMG-CoA reductase. Briefly, the binding enthalpies were obtained by injecting the statin into the microcalorimeter reaction cell containing the HMG-CoA reductase under stirring conditions. In a typical ITC experiment, the heat of reaction was obtained by making 30 injections (10 μL each) of the statin [final concentration in the syringe of 300 μM, in 20 mM Tris-HCl (pH 8.0), 2 mM TCEP, 1 mM NADPH, and 2% DMSO] into the reaction cell that contained HMG-CoA reductase in the same buffer (final protein concentration of 20 μM). The presence of a linked protonation−deprotonation reaction was checked by repeating titrations in phosphate buffer, a buffer with an ionization enthalpy (1.2 kcal/mol) significantly lower than that of Tris (11.3 kcal/mol) (*31*). The heat evolved after each injection was obtained from the time integral of the calorimetric signal using Origin 5.0, and the data were analyzed as described previously (*32*).

RESULTS AND DISCUSSION

Enzyme Characterization. HMG-CoA reductase catalyzes the conversion of HMG-CoA into mevalonate using NADPH as a cosubstrate according to the reaction

$$\text{HMG-CoA} + 2\text{NADPH} + 2\text{H}^+ \rightarrow \text{mevalonate} + 2\text{NADP}^+ + \text{CoASH}$$

The statins are competitive inhibitors with respect to HMG-CoA but not NADPH (*14*). The statins bind to the HMG-CoA reductase active site inhibiting the binding of the substrate due to its much higher affinity. The catalytic activity of the recombinant soluble domain of HMG-CoA reductase was evaluated by performing enzyme velocity measurements with respect to the two substrates, HMG-CoA and NADPH. For K_m and V_{max} determinations for both substrates, enzymatic assays were performed as described in Materials and Methods by varying the HMG-CoA or NADPH concentration in each case. Nonlinear least-squares analysis of the experimental values with respect to the Michaelis−Menten equation yielded K_m values of 70 and 21 μM for HMG-CoA and NADPH, respectively (Figure 2). These values are in agreement with those reported previously (*14*).

Enzyme Inhibition Experiments. The inhibition constant, K_i, for each statin was determined by measuring the decrease in catalytic activity with an increase in inhibitor concentration. Figure 3 shows the ratio of the initial enzyme velocity in the presence and absence of the statin as a function of the statin concentration for experiments performed at 37 °C. K_i's were determined by nonlinear least-squares fitting of the data to the standard equations for competitive inhibition for tight ligands (*30*, *33*). The resulting K_i values are listed in Table 1. Rosuvastatin and cerivastatin were found to have the highest potency (2.3 and 5.7 nM, respectively), while pravastatin and fluvastatin had the lowest potency (103 and 256 nM, respectively). Atorvastatin had an intermediate inhibitory potency (14 nM). These values are of the same magnitude as those reported previously (*23*, *34*).

Isothermal Titration Calorimetry. Isothermal titration calorimetry (ITC) experiments were performed in triplicate at three different temperatures (15, 25, and 35 °C). Figure 4 shows ITC experiments performed at 25 °C with pravastatin, cerivastatin, atorvastatin, and rosuvastatin. The binding thermodynamics of fluvastatin could not be measured by ITC due to extremely small reaction heats, suggesting that the binding enthalpy of this statin is very close to zero. Increasing the temperature to 35 °C did not improve the situation since the heat capacity change (temperature dependence of the enthalpy change) was also very small. As expected, the calorimetric titrations of the most potent statins (rosuvastatin, cerivastatin, and atorvastatin) resulted in stoichiometric titrations that provide accurate values for the binding enthalpy but only a lower limit for the binding affinity (*35*). Only in the case of pravastatin could the binding affinity be determined by ITC. The value for the binding affinity at 37 °C is 1.04×10^7 M^{-1} (K_d = 96 nM) which is very close to the inhibition constant (103 nM) measured spectroscopically. The Gibbs energies [$\Delta G = -RT \ln K_a = -RT \ln(1/K_d)$]

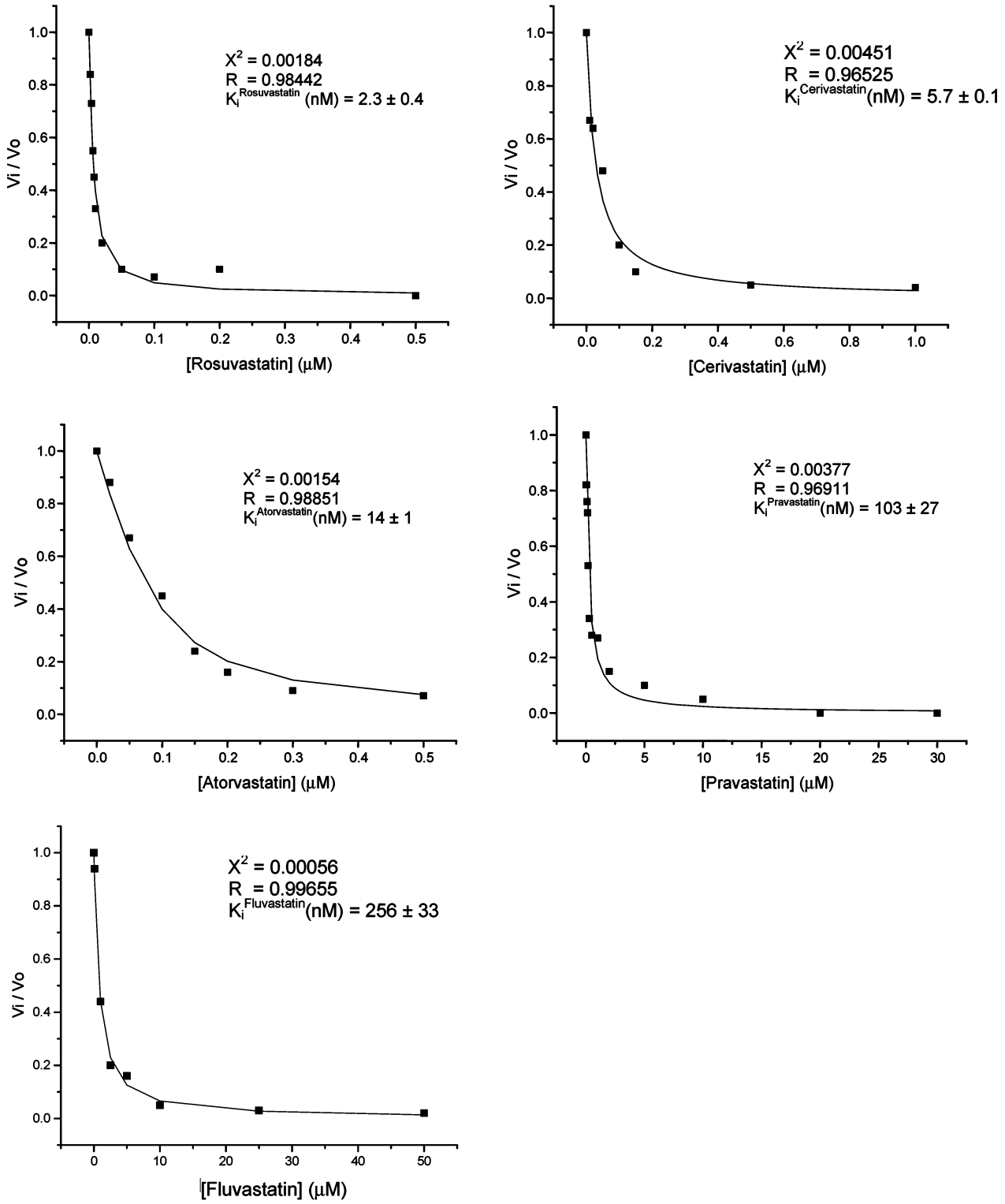

FIGURE 3: Determination of the inhibition constant, K_i, for the statins considered in these studies. Here, the normalized initial velocities (V/V_0) are plotted as a function of the statin concentration. V_0 is the initial velocity in the absence of inhibitor. The data were analyzed by nonlinear least squares in terms of the competitive inhibition model.

Table 1: Inhibition Constants for Statins at 37 °C

statin	K_i (nM)	statin	K_i (nM)
rosuvastatin	2.3 ± 0.4	pravastatin	103 ± 27
cerivastatin	5.7 ± 0.1	fluvastatin	256 ± 33
atorvastatin	14 ± 1		

obtained from ITC and inhibition assays were −9.96 and −9.90 kcal/mol, respectively. The Gibbs energies for rosuvastatin, cerivastatin, and atorvastatin were calculated from the inhibition constants.

With the possible exception of fluvastatin, the binding of all statins is characterized by favorable binding enthalpies ranging from −2.5 to −9.3 kcal/mol at 25 °C. Rosuvastatin exhibits the strongest binding enthalpy. Additional calorimetric titrations in phosphate buffer (not shown) were performed with rosuvastatin to check for the presence of any

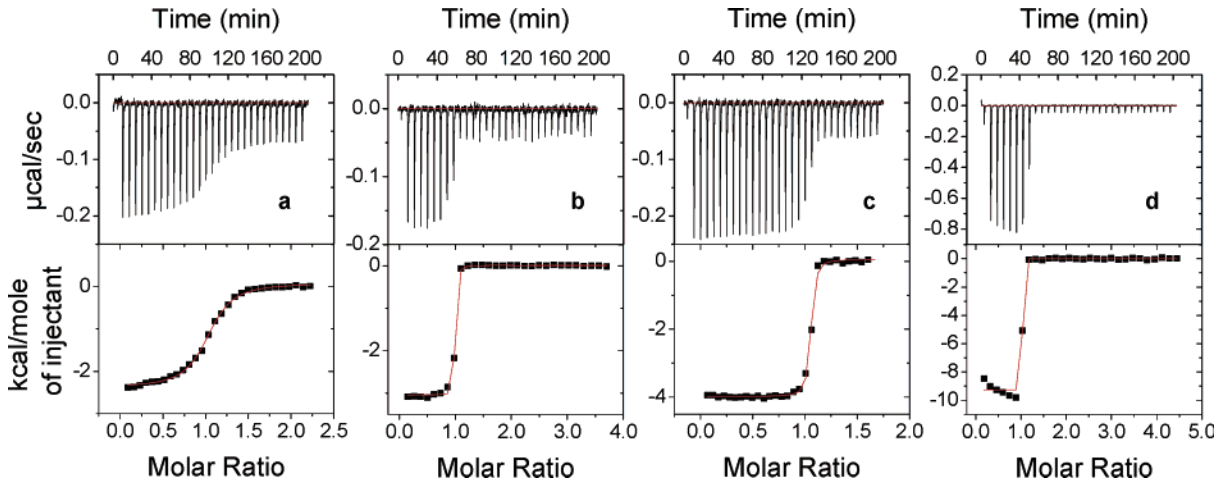

FIGURE 4: Calorimetric titrations of HMG-CoA reductase with pravastatin (a), cerivastatin (b), atorvastatin (c), and rosuvastatin (d). The experiments were performed in triplicate at 25 °C in 20 mM Tris-HCl (pH 8.0), 2 mM TCEP, 1 mM NADPH, and 2% DMSO.

Table 2: Thermodynamic Parameters Associated with Binding of Statin to HMG-CoA Reductase[a]

statin	ΔG (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)	ΔC_p (kcal K^{-1} mol^{-1})
fluvastatin	-9.0 ± 0.4	~ 0[b]	~ -9.0	ND[c]
pravastatin	-9.7 ± 0.4	-2.5 ± 0.1	-7.2 ± 0.4	-0.14 ± 0.02
cerivastatin	-11.4 ± 0.4	-3.3 ± 0.2	-8.1 ± 0.4	-0.15 ± 0.02
atorvastatin	-10.9 ± 0.8	-4.3 ± 0.1	-6.6 ± 0.6	-0.17 ± 0.05
rosuvastatin	-12.3 ± 0.7	-9.3 ± 0.1	-3.0 ± 0.7	-0.46 ± 0.04

[a] The values for ΔG, ΔH, and $-T\Delta S$ are quoted at 25°C. [b] The binding enthalpy of fluvastatin was beyond the detection limit of the instrument, suggesting that is close to zero, in which case most of the binding energy is entropic in origin. The free energy of binding was derived from enzyme inhibition assays. [c] Not determined.

coupled protonation−deprotonation reaction that might be influencing the measured binding enthalpy. The results were essentially the same within the experimental error, and indicated that at most only 0.1 proton was involved in the reaction under the conditions used in the experiments. At 37 °C, the binding enthalpy becomes even more favorable as the binding reaction is characterized by a negative change in heat capacity (Table 2). At 37 °C, the binding enthalpy of rosuvastatin is −14.8 kcal/mol, very close to the value of −16.6 kcal/mol previously reported by Holdgate (*34*). At this temperature, the binding enthalpies of pravastatin, cerivastatin, and atorvastatin are −4.2, −5.1, and −6.3 kcal/mol, respectively.

Thermodynamic Dissection of Binding Affinity. The proportion by which the binding enthalpy contributes to the binding affinity is not the same for all statins as illustrated in Figure 5. At 25 °C, for fluvastatin, pravastatin, cerivastatin, and atorvastatin the dominant contribution to the binding affinity is the entropy change. Only for rosuvastatin does the enthalpy change contribute more than 50% of the total binding energy (76%). At 37 °C, the differences are accentuated by the larger ΔC_p of rosuvastatin. At this temperature, the binding enthalpy contributes close to 100% of the binding energy of rosuvastatin and only 42, 44, and 57% for pravastatin, cerivastatin, and atorvastatin, respectively.

The differences in the proportion by which the enthalpy and entropy changes contribute to the binding affinity reflect differences in the type of interactions established between the various statins and HMG-CoA reductase. Since the crystal structures of the complexes with rosuvastatin, atorvastatin, cerivastatin, and fluvastatin are known (*23*), it is possible to analyze the binding thermodynamics of these statins in terms

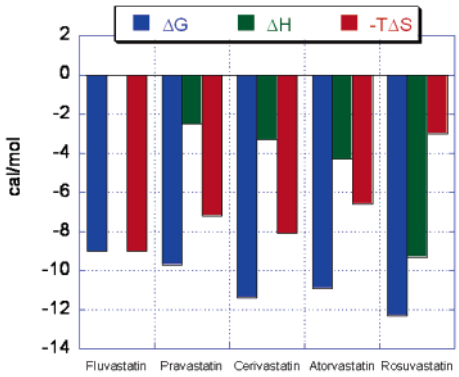

FIGURE 5: Proportion by which the binding enthalpy contributes to the Gibbs energy of binding for each of the statins that was studied.

of structure. All statins target the same site in the protein and according to the crystallographic structures do not cause differential conformational rearrangements in the enzyme that may significantly influence the binding energetics. Accordingly, differences in binding energetics can be attributed to different inhibitor−enzyme interactions. Furthermore, since the HMG region is the same in all inhibitors included in these studies, differences can be attributed to the so-called "hydrophobic region".

The binding enthalpy primarily reflects the strength of the interactions (hydrogen bond and van der Waals) between inhibitor and enzyme relative to those with water. For inhibitors targeted to the same site with no differences in coupled protonation−deprotonation reactions, the binding enthalpy truly reflects the strength of the inhibitor−enzyme interaction. The major favorable contribution to the binding entropy, on the other hand, originates from the release of

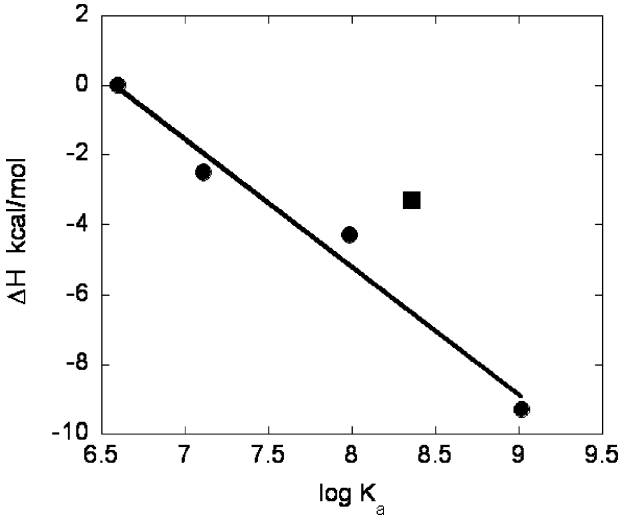

FIGURE 6: Statistical correlation between the binding affinity and the binding enthalpy for the statins. Except for cerivastatin (■), there is a very good correlation ($R = 0.976$) between the binding enthalpy and the logarithm of the binding affinity.

water molecules associated with the burial of hydrophobic surfaces upon binding (hydrophobic effect). This effect reflects the repulsion of the inhibitor from the solvent rather than an attractive inhibitor−enzyme interaction. As such, it is intrinsically nonspecific and acquires specificity only by combining it with shape complementarity of the inhibitor and binding cavity. Another source of binding entropy is related to changes in conformational degrees of freedom. As these changes usually involve fixing the inhibitor and certain regions of the protein in more restricted conformations, they lead to a diminished conformational entropy and therefore an unfavorable contribution to the binding affinity. This loss in conformational entropy is minimized, however, by conformationally constraining and preshaping the inhibitor to its bound conformation. Other entropy contributions such as those associated with translational degrees of freedom are the same for all inhibitors and do not contribute to differences in binding affinity.

As noticed earlier for HIV-1 protease inhibitors (*36*, *37*), there is a good correlation between binding affinity and binding enthalpy, indicating that very high affinity requires strong enthalpic interactions. Figure 6 shows the correlation between the binding affinity and binding enthalpy of the statins. Except for cerivastatin, the correlation is very good and characterized by a correlation coefficient R of 0.976. The deviation observed for cerivastatin indicates that this statin gains affinity by a larger than average entropy gain. In theory, the binding affinity can be optimized by improving the binding entropy, the binding enthalpy, or a combination of both. In practice, however, extremely high affinity is achieved when the enthalpy change is optimized since the entropic optimization, once conformational constraints are exhausted, is usually associated with increased hydrophobicity and eventually results in compounds with little or no water solubility.

Structure-Based Thermodynamic Analysis. The crystallographic structures of HMG-CoA reductase with the four type II statins considered in this study have been determined (*23*). All these inhibitors share the same HMG moiety (red in Figure 1) which establishes nonpolar as well as several polar interactions with the enzyme. These interactions are similar for all statins. The hydrophobic regions of the inhibitors (black in Figure 1) determine to a large extent their different thermodynamic behavior. They differ in the presence of different functional groups, their size, the number of carbon atoms, and the number and type of polar atoms. Upon binding, atoms from the inhibitor as well as the binding cavity become buried from the solvent. Table 3 summarizes the changes in solvent accessible surface area (ASA) for each complex. It is clear that not all statins bury the same polar and nonpolar surface when they bind to HMG-CoA reductase. The binding of atorvastatin and especially rosuvastatin results in the burial from the solvent of an amount of polar surface area (323 and 341 Å^2, respectively) significantly larger than that of fluvastatin and cerivastatin (291 and 304 Å^2, respectively). Fluvastatin and cerivastatin with hydrophobic regions of comparable dimensions bury similar amounts of nonpolar surface area (593 and 605 Å^2, respectively). Atorvastatin, with a much larger hydrophobic region, buries an additional ~100 Å^2 of nonpolar surface area (692 Å^2). On the other hand, rosuvastatin with the smallest hydrophobic region only buries 512 Å^2 of nonpolar surface area.

These structural features of the statins are consistent with the measured thermodynamic parameters. As expected, atorvastatin and especially rosuvastatin exhibit the strongest binding enthalpies (*38*). These two statins are also the only ones which establish a hydrogen bond between the so-called hydrophobic region and the enzyme. The carbonyl oxygen of atorvastatin forms a hydrogen bond (2.79 Å) with the hydroxyl group of Ser565, while the sulfonyl group in rosuvastatin forms a strong hydrogen bond (2.67 Å) with the hydroxyl of Ser565. The stronger binding enthalpy of rosuvastatin appears to originate from the combination of a shorter bond length and the atom types involved. We have observed experimentally for other protein systems that ligands with sulfonyl functionalities participating in hydrogen bonds with the protein are also characterized by strong favorable binding enthalpies (ref *37* and unpublished observations from this laboratory). The heat capacity change associated with the binding of rosuvastain (ΔC_p) is also significantly larger than that of the other statins (Table 2). As in the case of the binding enthalpy, we and others have also observed larger heat capacities for binding processes involving sulfonyl groups that become buried from the solvent and strongly hydrogen-bonded upon binding (ref *42* and unpublished observations from this laboratory). On the other hand, fluvastatin binds with a binding enthalpy close to zero while cerivastatin, which like fluvastatin does not participate in hydrogen bonding interactions beyond the HMG region, binds with a favorable enthalpy of −3.3 kcal/mol. Structurally, the nitrogen in the indole ring of fluvastatin is completely buried, while the oxygen and nitrogen atoms in the hydrophobic region of cerivastatin are partially exposed. Thermodynamically, fluvastatin pays a higher desolvation enthalpy penalty than cerivastatin. For an aromatic nitrogen, the enthalpy of desolvation can be as high as 5 kcal/mol (*39*) and can easily account for the insignificant binding enthalpy of fluvastatin.

Two factors are major contributors to the observed differences in binding entropy between statins, the burial of hydrophobic surfaces from the solvent and the loss of

Table 3: Structure-Based Thermodynamic Analysis of Binding of Statin to HMG-CoA Reductase at 25 °C

statin	PDB entry	ΔASA_{pol} (Å^2)	ΔASA_{np} (Å^2)	$-T\Delta S_{solv}$ (kcal/mol)	no. of H-bonds (hydrophobic region)	no. of rotatable bonds (hydrophobic region)
fluvastatin	1hwi	−291	−593	−17.5	0	4
cerivastatin	1hwj	−304	−605	−17.8	0	6
atorvastatin	1hwk	−323	−692	−20.5	1	9
rosuvastatin	1hwl	−341	−512	−14.3	1	6

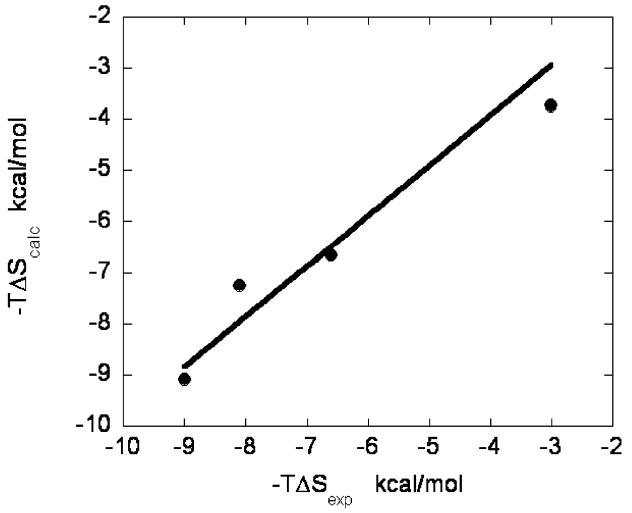

FIGURE 7: Correlation plot for the binding entropies calculated from the crystallographic structures and the experimental values. The solid line is the linear least-squares fit and is characterized by a zero intercept, a slope of 0.982, and a correlation coefficient of 0.958.

conformational degrees of freedom upon binding. The change in solvation entropy can be calculated in terms of the changes in ΔASA_{np} and ΔASA_{pol} as described previously (*40*). If this is the case, for the various statins the entropy change can be written as the sum of a constant term plus the conformational and solvation terms:

$$-T\Delta S_{calc} = c - T\Delta S_{solv} + dN_{rot} \quad (1)$$

where the conformational entropy term is assumed to be proportional to the number of rotatable bonds in the statin. Nonlinear regression analysis of the data yields a value of −0.44 kcal/mol for the constant c and a value of 1.1 kcal/mol as the effective contribution associated with each rotatable bond. This effective contribution includes changes in the statin molecule and those induced in the protein molecule. Figure 7 shows a correlation plot for the experimental and calculated binding entropies. Several conclusions can be derived from this analysis. As expected, atorvastatin shows the largest solvation entropy contribution to binding due to its much larger hydrophobic surface. The large contribution of this term to affinity is, however, compensated by the larger loss of conformational entropy associated with a larger number of rotatable bonds (Table 3). Overall, fluvastatin with fewer rotatable bonds shows the highest entropic contribution to binding. Cerivastatin exhibits a solvation entropy similar to that of fluvastatin but a lower overall entropic contribution due to the presence of two additional rotatable bonds. However, fluvastatin is a low-affinity inhibitor when compared to the other statins (Table 2). Among the high-affinity inhibitors, cerivastatin exhibits the highest entropy contribution to binding. While atorvastatin is the statin with the largest hydrophobic region and consequently the largest solvation entropy change, it has three more rotatable bonds than cerivastatin and, therefore, a larger conformational entropy loss that diminishes its entropic contribution below that of cerivastatin.

CONCLUSIONS

The statins are among the most widely used prescription drugs in the world. For drugs used by millions on a daily basis like the statins or COX-2 inhibitors, safety issues have taken on the outmost importance. Since many of these issues can be related to a lack of selectivity arising from nonspecific binding to unwanted targets, it is important to explore how these drugs bind to their intended targets, the nature of the forces that drive the binding reaction, and whether there are any correlations between their binding mechanism and their selectivity. Since the thermodynamic signature of an inhibitor reflects the types of interactions that drive the binding reaction, it can become an important tool for assessing the potential for a compound to associate nonspecifically with other proteins. Previously, we have shown that in the case of HIV-1 protease inhibitors there is a significant correlation between the thermodynamic signature of inhibitors and their susceptibility to mutations associated with drug resistance (*41*). The studies presented here represent a first attempt at determining the binding thermodynamics of an important class of drugs and provide fundamental information for understanding their binding mechanism.

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