DFT study on hydroxy acid–lactone interconversion of statins: the case of atorvastatin†

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Atorvastatin (ATV), the best known HMG-CoA reductase inhibitor family member, undergoes pH-dependent hydroxy acid–lactone interconversion similar to other statins. Although the only active form is a linear one, it was shown that drug interactions should also be considered for the lactone. The ATV lactonisation–hydrolysis mechanism was investigated theoretically using the density functional theory (DFT) method. Under both mildly acidic and basic conditions, the ATV lactone form is less stable than its hydroxy acid form. However, in the presence of a carboxylic acid, the equilibrium was only slightly shifted towards the lactone side (4 kcal mol^{-1} difference between the substrate and the product), while energy gain for the hydrolysis under basic conditions amounts to 18 kcal mol^{-1}. Hydrolysis activation energy barriers were 19 and 6 kcal mol^{-1}, in acidic and basic conditions, respectively. We determined one-step interconversion as unfavourable under physiological conditions due to a 35 kcal mol^{-1} activation energy barrier. All data were compared with analogue ones for fluvastatin (FLV) reported earlier and indicated that ATV is more flexible than FLV, not only due to the fact that it has more rotatable carbon–carbon single bonds, but also because ATV lactonistation–hydrolysis energy barriers are lower.

Introduction

Atorvastatin (ATV) (trade names Lipitor, Torvast, Sortis) has been the best selling drug since 2001. In 2006, annual sales of Lipitor were $13.6 billion.[1] Probably, Lipitor will be the highest selling prescription drug worldwide in 2007 as well. Atorvastatin (IU-PAC name 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-yl-pyrrol-1-y l]-3,5-dihydroxy-heptanoic acid, Fig. 1) belongs to the family of HMG-CoA reductase inhibitors also known as statins. Research into inhibitors of HMG-CoA reductase commenced in 1971. The first commercially marketed statin was Lovastatin isolated from the mould *Aspergillus terreus* in 1976.[2] The basic application of statins is a treatment of lipid disorders. They block the biosynthesis of cholesterol by inhibiting HMG-CoA reductase, which suppresses synthesis of endogenous cholesterol. Because the liver synthesises less cholesterol, the number of high affinity low-density lipoprotein (LDL) receptors increases. As a result LDL and cholesterol blood levels decrease.[3,4] Studies of the statins showed that they delay the progression of coronary artery disease,[5] reduce mortality from cardiovascular diseases[6] and have anti-inflammatory[7] and anti-oxidant activity.[8] There are some reports on testing HMG-CoA reductase inhibitors against cancer,[9] Alzheimer's disease, and osteoporosis.[10]

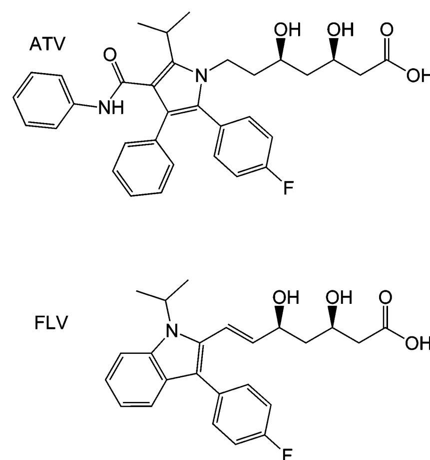

Fig. 1 Chemical structures of atorvastatin (ATV) and fluvastatin (FLV).

However, concerns about side effects are increasing, in particular due to the prolonged administration. In the last few years, the number of drugs and dietary supplements taken by an average person has been steadily increasing. Moreover, the mechanisms of action of most of them are not known as well as they should be. It is especially true for inter-drug interactions. Therefore, there is a need for further studies of the already developed drugs as well to supplement our knowledge about them, especially in the case of a drug as popular as atorvastatin.

Atorvastatin is a totally synthetic statin. Its molecule is composed of a lipophilic moiety containing four aromatic rings and a heptanoic acid side chain with a 3,5-diol moiety. It is administrated orally as the calcium salt but the labile 3,5-diol moiety undergoes reversible pH-dependent lactonisation.[11,12] At the physiological pH and higher, the lactone form is unstable and the equilibrium

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favours hydrolysis, which opens the lactone and yields the hydroxy acid form. The latter is susceptible under acidic conditions to lactone formation.[13,14] In general, the lactone and acid forms co-exist in the *in vivo* equilibrium and in the case of many statins, the lactone form is at least as abundant as the hydroxy acid.[15,16] Crystal structures clearly indicate that the linear forms of statins are predisposed for binding to the enzyme, as the terminal carboxylate group forms salt bridges with Lys692 and Lys735, while the δ-hydroxy group serves as a charge-assisted hydrogen bond donor to Glu559 and as a hydrogen bond acceptor from Lys691.[30] Although only the acid form lowers cholesterol levels, it has been shown that drug interactions should be considered for both acid and lactone forms.[17]

Recently, the mechanism of lactone–hydroxy acid interconversion in the case of fluvastatin (FLV), under both acidic and basic conditions, was investigated by us theoretically using the density functional theory (DFT) methods.[18] Regardless of the conditions, the lactone form of FLV was always higher in energy by 6 kcal mol^{-1} in acidic and 19 kcal mol^{-1} in basic conditions. However, under basic conditions, the activation barrier for the hydrolysis was significantly lower (9 kcal mol^{-1}) than for the reverse reaction (28 kcal mol^{-1}), making the lactone form highly unstable. The activation barriers under acidic conditions were of comparable value in both directions (22 kcal mol^{-1} for hydrolysis and 28 kcal mol^{-1} for lactonisation), making the occurrence of both forms probable. Due to the high activation barrier (>40 kcal mol^{-1}), a one-step, direct interconversion between the two forms turned out to be unfavourable. Moreover, relatively small energy differences (<5 kcal mol^{-1}) between the key conformers[18] were reported.

Although atorvastatin (ATV) and fluvastatin (FLV) belong to the same group of statin drugs, there are important differences between them (Fig. 1). The inhibition constant of HMG-CoA reductase is $IC_{50} = 27.6$ (nM) for fluvastatin and $IC_{50} = 8.2$ (nM) for atorvastatin.[19] Thus atorvastatin is a more efficient and more popular drug. However, atorvastatin does not contain the double carbon–carbon bond in the dihydroxy acid side chain, so there are more conformers possible.

In the current study, we focused on atorvastatin lactonisation–hydrolysis and compared the results with the analogue ones for fluvastatin as they may help us understand how the subtle differences in the structures of these molecules affect the activity of the drugs.

Computational details

All calculations presented in this report were performed with the Gaussian 03[20] and AMSOL[29] programs. The structures were built using standard bond lengths, valence angles and dihedrals. To facilitate calculations, the structures were pre-optimised at the HF/6-31G(d) level of theory. For further calculations, the popular Becke's hybrid functional with LYP[21,22] potential (B3LYP) was used. It was combined with the 6-31G(d) basis set. In 6-31G(d), each valence shell is split into two parts described by three and one Gaussian function, respectively. Inner shells are represented by a single basis function expressed as a sum of six Gaussians.[23] Moreover, a set of polarisation functions of d symmetry is present in non-hydrogen atoms. Transition state structures and local potential energy minima were characterised vibrationally to verify the presence of the correct number of imaginary frequencies (one for the transition states, zero for the minima). We also optimised at this level of theory the structure of ATV observed in the crystal structure in complex with HMG-CoA reductase (PDB refcode 1HWK).[30] Moreover, IRC calculations were performed to verify which potential energy minima are connected by a given TS. The energies of all optimised structures were finally calculated using the B3LYP method combined with a 6-31+G(d) basis set (6-31G(d) augmented with diffuse function), because it was shown that at least a single set of diffuse functions should be used for the proper description of the ionic system.[24–27]

The solvation free energies in aqueous media were calculated using SM5.4 model with AM1 and PM3[28] Hamiltonians implemented in the AMSOL package.[29] Briefly, the solvation models (SMx) are semiempirical models that introduce into calculations the effects of various solvents. In the SM5.4 terms responsible for cavity formation, dispersion, solvent structure and local field polarisation are present. The solvation energy is obtained *via* the usual approximation that solute treated at the quantum mechanical level is immersed in an isotropic, polarisable continuum representation of a solvent. The semiempirical SMx methods have two major advantages. First, they make up for errors intrinsic to replacing a continuous charge distribution by a set of distributed point charges because the mapping from which they are obtained is chosen to minimise errors in the physical observables predicted from point charges. Second, they make up for deficiencies in the semiempirical wave function from which they are obtained because the parametrisations are chosen to minimise deviations from experiment.

The hydration free energies calculated with SM5.4A and SM5.4P methods were added to energies of the isolated structures calculated at B3LYP level to gain insight into the relative stability of these structures in aqueous solution. For the sake of clarity, we report here only the results from SM5.4A calculations as the SM5.4P results were essentially the same and are presented as supplementary material.†

Results and discussion

Mechanism of atorvastatin hydroxy acidic to lactone interconversion under acidic conditions

The kinetics of interconversion and the equilibrium between the hydroxy acid and the lactone form as a function of pH studied experimentally showed that the acid-catalysed reaction is reversible.[14] At pH < 6, an equilibrium favouring the hydroxy acid form was established.[14] It is well known that the reaction mechanisms studied computationally with H_3O^+ (not mentioning those using an isolated proton) show very low energy barriers for isolated species.[31,32] Some studies indicate that the use of uncharged groups as the source of protons results in more realistic interconversion barriers.[33–35] Therefore, we decided to use carboxylic acid as a source of protons. We chose formic acid as the simplest one and also to provide the same mildly acidic conditions as reported earlier for fluvastatin.[18]

In the initial pre-reaction complex,[18] the formic acid forms hydrogen bonds with the acidic form of ATV as it accepts a hydrogen bond from the 5-OH group of ATV and donates another hydrogen bond to the carboxylic oxygen atom of ATV. We

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determined two possible reaction mechanisms starting from that point (Table 1, Fig. 2, Fig. 3). The first one (**A-AL-L**) is a one-step process with a very high energy barrier over 35 kcal mol^{-1} (above the starting structure of the pre-reaction complex). More specifically, there are two possible transition states which differ from each other in protonation patterns, *i.e.* in **AL1** (38 kcal mol^{-1} for the isolated system and 37 kcal mol^{-1} in aqueous solution), the leaving water molecule is in the axial position with respect to the forming lactone ring, while in **AL2** (36 kcal mol^{-1} for the isolated system and 35 kcal mol^{-1} in aqueous solution), the water molecule is in the equatorial position. Under physiological conditions, such high energy barriers are not likely to be possible.

Further investigations led us to the second reaction mechanism. It goes through four transition states and requires 20 kcal mol^{-1} less energy. In the first step, the carbonyl oxygen atom is protonated. At the same time, the proton dissociates from the 5-OH group and the oxygen atom attacks the carboxylic carbon atom (**TS**, 16. kcal mol^{-1} for the isolated system and in aqueous solution). The ring closes and a hydrated lactone is formed (**II**, 6 kcal mol^{-1}). On the way to the lactone form, the hydrated lactone eliminates one water molecule, which may leave the newly formed lactone ring in the axial or equatorial positions. Therefore, two structures (**IV_ax** and **IV_eq**), which differ from each other by protonation patterns, may be formed from **II**, by passing two TS (**TS_1**, **TS_2**) in which the rotation of terminal OH groups takes place. As we can see (Fig. 2), there is a significant energy difference between states **TS2_ax** (18.4 kcal mol^{-1} for the isolated system and 16.0 kcal mol^{-1} in aqueous solution) and **TS2_eq** (5.9 kcal mol^{-1} for the isolated system and 4.4 kcal mol^{-1} in aqueous solution). Therefore, it may seem that the reaction goes mainly through **TS1_eq** and **TS2_eq** transition states. Probably, it is not so. The next step (**TS3_eq**) requires 25 kcal mol^{-1} energy (both in aqueous solution and *in vacuo*) compared to 18.7 kcal mol^{-1} in the case of **TS3_ax**, which can be easily explained by the 1,3 diaxial interactions. None of the "**ax**" transition states is higher than 19 kcal mol^{-1}. When this level of energy is achieved in the initial process, there are no additional energy barriers to overcome. Among the lactone forms created, the one with the lowest potential energy (**V_ax**) is energetically favoured by 4.2 kcal mol^{-1} over the isolated substrates (4.7 kcal mol^{-1} in aqueous solution). The

Table 1 Relative energies (in kcal mol^{-1}) of structures present during ATV hydroxy acid to lactone interconvertion under acidic conditions calculated for isolated species and in aqueous solution

Structure	ΔE (isolated)	ΔG SM5.4A hydration	ΔE (solution)
I	0.0	−8.8	0.0
A_AL2	4.1	−10.0	2.8
A_AL1	11.3	−9.5	10.6
TS	16.5	−9.1	16.3
AL2	35.8	−10.1	34.5
AL1	37.8	−9.2	37.3
II	6.0	−8.4	6.4
TS1_eq	6.2	−8.4	6.6
TS1_ax	8.8	−8.5	9.1
III_eq	5.1	−8.9	5.0
III_ax	6.1	−9.3	5.6
TS2_eq	5.9	−10.3	4.4
TS2_ax	18.4	−11.2	16.0
IV_eq	4.5	−10.4	2.9
IV_ax	5.7	−9.6	4.8
ST3_eq	25.1	−9.4	24.5
TS3_ax	18.7	−9.2	18.3
L_AL2	−1.7	−8.7	−1.6
L_AL1	−4.2	−9.3	−4.7
V_eq	−1.7	−8.7	−1.6
V_ax	−4.2	−9.3	−4.7

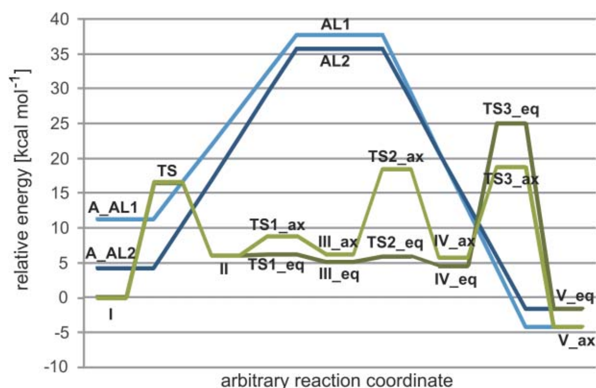

Fig. 2 Reaction pathways for the interconversion between the acid (A \ I) and lactone (V) forms of ATV under mildly acidic conditions.

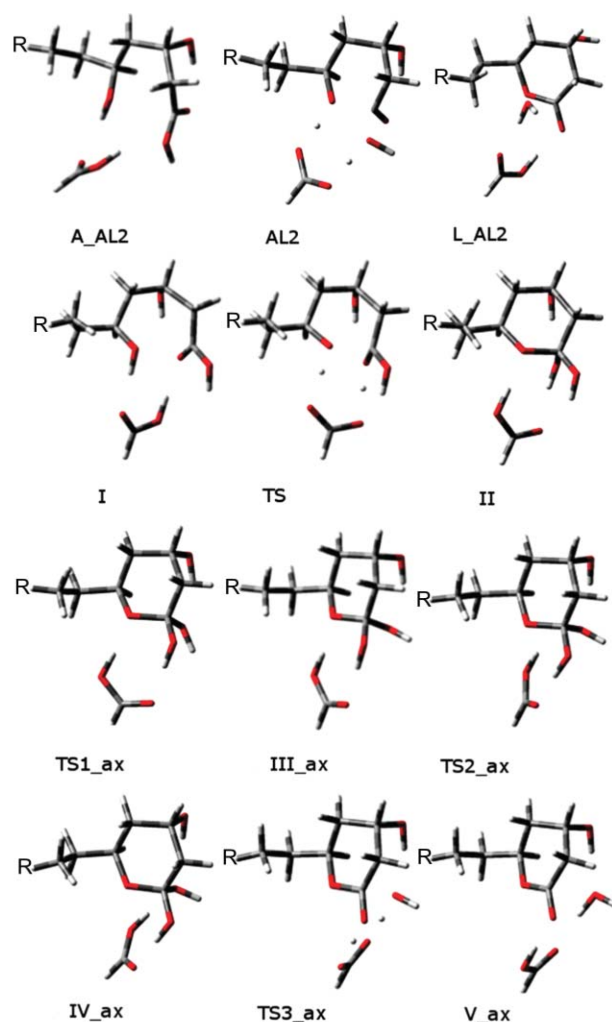

Fig. 3 Structures of selected stationary points along ATV interconversion pathways under acid conditions. R – represents a bulky hydrophobic substituent which was included in the calculations, but for clarity is not presented in the figure.

reaction leading from the acid form of ATV to the lactone form is thus exoergic and the energy barrier for this conversion is about 19 kcal mol^{-1}. The reverse, endoergic reaction has a slightly higher activation energy barrier of 23 kcal mol^{-1}. Hydroxy acid–lactone interconversion equilibrium in the presence of formic acid conditions favours the lactone form, which is in line with the experimental findings.

As expected, the differences between ATV and FLV in the lactonisation–hydrolysis reaction are fine. Nevertheless, the activation barrier for lactone formation is smaller for ATV (19 kcal mol^{-1}) than for FLV (22 kcal mol^{-1}). Thus, differences in the structure of the hydrophobic moiety seem to have only limited influence on the hydroxy acid chain.

Mechanism of the transition from the lactone form (L) to the carboxylate salt (S) of ATV under basic conditions

From our calculations, the equilibrium of ATV lactonisation–hydrolysis under basic conditions is shifted towards the carboxylate salt (Table 2, Fig. 4), which is in agreement with the experimental findings.[13,14] The energy gain upon lactone hydrolysis amounts to 21 kcal mol^{-1} for the isolated system and 18.1 kcal mol^{-1} in aqueous solution (Fig. 5).

The reaction *in vacuo* goes through two transition states, **LO** and **OS**, with relative energies of 26.9 and 23.9 kcal mol^{-1}, respectively. In the **LO** transition state, a hydroxy anion attacks the lactone ring (**L**) and hydrated lactone (**O**) is formed. For the isolated system, the hydrated lactone (**O**) corresponds to the shallow, potential energy local minimum nearly 22.9 kcal mol^{-1} higher than that for the ATV carboxylate. Subsequently, the bond between the oxygen and the carboxylic carbon is hydrolysed (**OS**), and the ATV carboxylate anion interacting with a water molecule is formed. However, in aqueous solution, the shallow minimum **O** does not exist, so the only transition state corresponds to the structure **OS**, whose relative energy with respect to the carboxylate anion (**S**) is 27.9 kcal mol^{-1}. Therefore, the activation energy for lactone hydrolysis amounts to 5.9 kcal mol^{-1} for the isolated system and 9.8 kcal mol^{-1} in aqueous solution. Moreover, the activation energy barrier of the reverse reaction in basic conditions is nearly 28 kcal mol^{-1}. Generally, the lactone form under basic conditions is highly unstable and the reaction proceeds towards the carboxylate salt.

Table 2 Relative energies (in kcal mol^{-1}) of structures present during ATV lactone hydrolysis under basic conditions calculated for isolated species and in aqueous solution

Structure	ΔE (isolated)	ΔG SM5.4A hydration	ΔE (solution)
S	0.0	−48.8	0.0
OS	23.9	−44.8	27.9
O	22.9	−47.9	23.8
LO	26.9	−56.7	22.7
L	21.0	−55.4	18.1

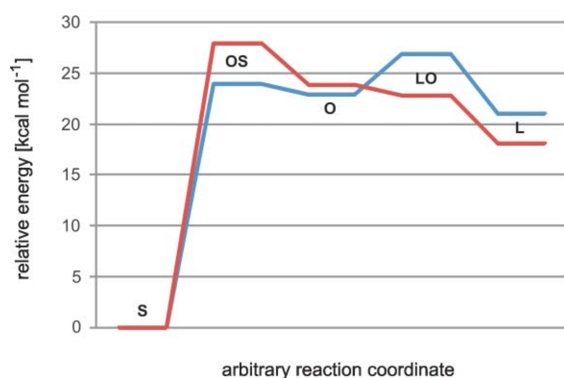

Fig. 4 Reaction pathways for the interconversion between the linear (S) and lactone (L) forms of ATV under basic conditions. The blue line refers to the process between isolated species, while the red line refers to the process in aqueous solution. The shallow potential energy minimum (O) present for the isolated species disappears when the aqueous solution is taken into account in calculations.

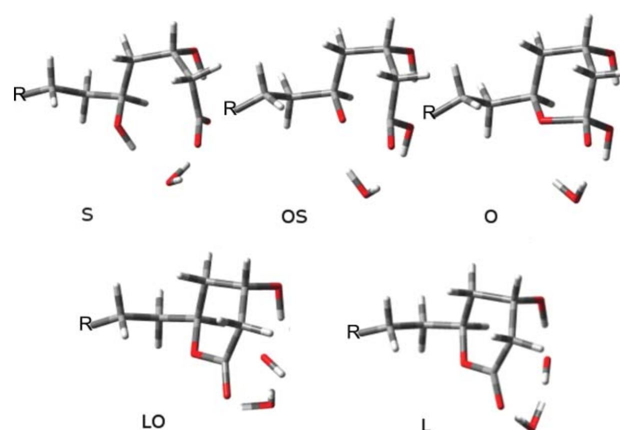

Fig. 5 Reaction pathway, under basic conditions, for the interconversion between the linear anionic form of ATV interacting with water molecule (S), and the lactone form (L) together with water molecule and hydroxide anion. R – represents a bulky hydrophobic substituent which was included in the calculations but for clarity is not presented in the figure.

The data for FLV also indicated that the lactone form was unstable in basic conditions. Nevertheless the hydrolysis activation barrier is significantly lower for ATV (6 kcal mol^{-1}) than for FLV (10 kcal mol^{-1}).

Conclusions

The calculations presented in this report, based on the density functional theory (DFT), fully support the previously reported findings on the pH-dependent character of atorvastatin hydroxy acid–lactone interconversion. Four reaction pathways connecting both forms were found and analysed. Two one-step processes observed under acidic conditions are characterised by high activation energy barriers at a level over 35 kcal mol^{-1}, which makes these pathways unlikely under physiological conditions. However, other reaction pathways with significantly lower activation energy barriers were also found. They go through an activation energy barrier of *ca.* 19 kcal mol^{-1}. In fact this reaction path can be split into two different ways, depending on protonation patterns. Energy reasons favour the leaving water molecule in the axial position with respect to the forming lactone ring. The overall equilibrium is slightly shifted towards the lactone side of the reaction as the lactonisation reaction is exoergic (about 4 kcal mol^{-1}).

Under basic conditions the hydroxy acid form is much more stable. In the hydrolysis reaction the energy gain is large, amounting to 18 kcal mol^{-1}. The activation energy barrier is less than

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10 kcal mol^{-1} while for the reverse reaction (lactone formation), it nearly amounts to 28 kcal mol^{-1}. Thus under basic conditions, atorvastatin in the molecular anion form is strongly energetically favoured. Our results suggest that in basic conditions, the lactone form of ATV is even less stable than FLV, as activation energy barriers for hydrolysis are 6 and 10 kcal mol^{-1} for ATV and FLV respectively. Also in the mildly acidic conditions the energy span of the lactonisation reaction was slightly smaller for ATV (*ca.* 19 kcal mol^{-1}) than for FLV (22 kcal mol^{-1}), presumably due to the fact that the dihydroxy acid side chain of ATV is more flexible. All in all, the ATV molecule seems to be more flexible than FLV—contrary to FLV, it does not possess a carbon–carbon double bond in the dihydroxy acid side chain. But also, thanks to lower activation barriers for lactonisation and hydrolysis reactions, the ATV molecule can adopt more chemical structures more easily than FLV.

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