Molecular mechanism for inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase by rosuvastatin

G.A. Holdgate, W.H.J. Ward1 and F. McTaggart
AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

Abstract
The statins are inhibitors of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (HMG-CoAR), and are used to decrease levels of atherogenic lipoproteins in patients with, or who are at high risk of, cardiovascular disease. This study describes the inhibition of a recombinant, catalytic fragment of human HMG-CoAR by a new statin, rosuvastatin (CRESTOR®). Binding is reversible and involves an initial complex (inhibition constant involving the enzyme–inhibitor complex (E.I), \( K_i \approx 1 \text{ mM} \)), which undergoes a slow transition (\( t_s \), to reach steady state is 33–360 s) to give tighter association [steady-state inhibition constant involving E.I and the second E.I complex in a two-step mechanism (E.I+), \( K_i^* \approx 0.1 \text{ mM} \)]. At steady state, rosuvastatin is at least as potent as atorvastatin, cerivastatin and simvastatin. It is more potent than fluvastatin and pravastatin. For rosuvastatin, inhibition kinetics are competitive with respect to HMG-CoA and non-competitive when NADPH is varied. At 37 °C, binding is linked to a large favourable enthalpy change \([\Delta H = -69.0 \text{ kJ/mol (~16.5 kcal/mol)}]\) and a small entropic penalty \([\Delta S = -9.6 \text{ kJ/mol (~2.3 kcal/mol)}]\). These characteristics, and the high affinity relative to that of 35-HMG-CoA (\( K_d \approx 6.6 \mu\text{M} \)), are discussed in relation to the crystal structures of complexes with HMG-CoAR.

Introduction
Elevated plasma cholesterol, especially low-density lipoprotein (LDL) cholesterol, is linked to an increased risk of morbidity and mortality in atherosclerotic diseases (angina, heart attack, stroke and peripheral vascular disease). In humans, most cholesterol biosynthesis occurs in the liver. The enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMG-CoAR; E.C 1.1.1.34) is responsible for catalysing the NADPH-dependent, two-step reduction of HMG-CoA to mevalonate (Figure 1). This reaction is a highly regulated process within the cholesterol biosynthetic pathway, and so is an attractive target for intervention in the treatment of hypercholesterolaemia. A decrease in cholesterol synthesis in cells leads to a homoeostatic response, involving up-regulation of cell-surface receptors that bind atherogenic lipoproteins such as LDL and very-low-density lipoprotein [1]. Bound lipoproteins are taken up into cells and degraded [2]. This reduction in circulating atherogenic lipoproteins helps to explain the clinical utility of HMG-CoAR inhibitors [3].

Human HMG-CoAR is a transmembrane glycoprotein, situated on the endoplasmic reticulum. Over 330 residues towards the N-terminus are membrane-associated. Residues 340–459 form a linker to the soluble C-terminal catalytic region (residues 460–888) [4]. A recombinant catalytic fragment, HMG-CoAR(426–888), crystallizes as a tetramer, which is effectively a dimer of dimers, with the four active sites located at monomer interfaces. The binding site for HMG-CoA is predominantly on one monomer, which is adjacent to the NADPH-binding site on another monomer. A recent publication describes the three-dimensional structures of human HMG-CoAR in complexes with six different statins [5]. The enzyme-catalysed reaction (Figure 1) probably involves His-866 and Glu-559 both donating protons [4]. The role of Glu-559 appears to be facilitated by the proximity of Asp-767, which may elevate the \( pK_a \) of the carboxylate so that a higher proportion is protonated at physiological pH.

Several HMG-CoAR inhibitors have been developed as cholesterol-lowering therapeutic agents. Known as statins, they are administered either as salts of carboxylic acids or as lactones, which undergo a ring-opening reaction to generate the inhibitory acids in vivo [6]. In part, the inhibitory acids have structural similarity to the tightly bound catalytic intermediate, mevaldyl-CoA (Figure 1). Rosuvastatin (CRESTOR®) is a new statin, originally identified and developed by Shionogi and Company. In assays that measure cholesterol biosynthesis, it is a more potent inhibitor than several other statins in rat hepatocytes (\( IC_{50} = 0.2 \text{ nM} \), compared with 1.2–6.9 nM for atorvastatin, simvastatin, cerivastatin, fluvastatin and pravastatin) [7]. Rosuvastatin is taken up selectively into the liver after intravenous administration to the rat, where it has a prolonged
Figure 1 | Catalysis by HMG-CoAR and inhibition by rosuvastatin
Top panel: the reaction catalysed by HMG-CoAR. Bottom panel: the structure of rosuvastatin and kinetic scheme for inhibition [9–11]. There is rapid formation of an initial E.I complex, with a dissociation constant, \( K_i \approx 1 \text{nM} \), followed by a slow transition (forward rate constant \( k_f = 0.019 \text{s}^{-1} \); reverse rate constant \( k_r = 0.009 \text{s}^{-1} \)) to give a more tightly bound complex, and an overall steady-state inhibition constant, \( K_{i^*} \approx 0.1 \text{nM} \).

Structure of and kinetics of inhibition by rosuvastatin
Our enzyme activity assays on human HMG-CoAR(419–888) indicate that binding is reversible and involves an initial complex [inhibition constant involving the enzyme–inhibitor complex (E.I), \( K_i \approx 1 \text{nM} \)], which undergoes a slow transition to give tighter association [steady-state inhibition constant involving E.I and the second E.I complex in a two-step mechanism (E.I*), \( K_{i^*} \approx 0.1 \text{nM} \)], to reach steady state is 33–360 s, depending on the concentrations of rosuvastatin and HMG-CoA] (see [9] and Figure 1). Inhibition kinetics are competitive with respect to HMG-CoA and non-competitive when NADPH is varied. Similar slow binding kinetics have been reported for compactin [10], lovastatin, fluindostatin and analogues [11].

The crystal structures of human HMG-CoAR(426–888) in complexes with statins [5] are consistent with the observed kinetics of inhibition. Rosuvastatin occupies a region where the hydroxymethylglutaryl moiety of the substrate binds, and it does not fill any of the NADPH site. Each bound inhibitor molecule interacts with two subunits of the HMG-CoAR tetramer. Several groups make multiple hydrogen bonds with the protein. In all complexes with statins, there is no visible electron density after Gly-860 to the C-terminus of the protein. These residues form helix Lo11 in a complex containing HMG, CoA and NADP+ [12].

The disorder in complexes with statins is required to expose a groove, which binds the hydrophobic moieties of the inhibitors [5].

The structural basis of the two-step binding process is not clear. The forward and reverse rate constants for the equilibrium between E.I and E.I* may lead to changes in the degree of inhibition during the time scale of enzyme assays, but they are sufficiently rapid to be unlikely to influence the pharmacokinetics and biological activity of rosuvastatin. The magnitudes of the forward and reverse rate constants are similar for rosuvastatin (Figure 1), lovastatin, fluindostatin and analogues [11]. The increase in affinity in going from E.I to E.I* appears to be comparable in magnitude.
Table 1 | Inhibition constants for rosuvastatin and other statins

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Initial $K_i$ (nM)</th>
<th>Steady-state $K_i^*$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>160 (110–220)</td>
<td>3.5 (2.5–5.0)</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>130 (93–190)</td>
<td>8.0 (6.0–12)</td>
</tr>
<tr>
<td>Cerivastatin</td>
<td>ND</td>
<td>10 (7.0–15)</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>ND</td>
<td>11 (7.0–17)</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>ND</td>
<td>28 (18–42)</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>ND</td>
<td>44 (29–66)</td>
</tr>
</tbody>
</table>

Figure 2 | Binding thermodynamics for HMG-CoA and rosuvastatin

Measurements were made at 37°C by isothermal titration calorimetry [13]. 1 kcal = 4.184 kJ.

for various statins (Table 1). These observations suggest that the transition from E.I to E.I$^*$ may reflect a common change in the conformation of the enzyme.

Thermodynamics of binding of rosuvastatin

Enzymes bind catalytic intermediates more tightly than substrates. The structure of rosuvastatin resembles that of the intermediate, mevaldyl-CoA, more closely than that of HMG-CoA (Figure 1). The inhibitor associates over 10 000-fold more tightly with the enzyme than does the substrate, and some of the causes for this affinity are suggested by a combination of the crystal structures and data on the thermodynamics of binding (Figure 2). Binding of rosuvastatin is linked to a large favourable enthalpy change [$\Delta H^\circ = -69.0 \, \text{kJ/mol} (-16.5 \, \text{kcal/mol})$] and a small entropic penalty [$T\Delta S^\circ = -9.6 \, \text{kJ/mol} (-2.3 \, \text{kcal/mol})$], suggesting that association involves favourable bond formation and a limited increase in structural order. C-5 is tetrahedral for rosuvastatin and mevaldyl-CoA, whereas it is trigonal for the substrate. A C-5 hydroxyl on rosuvastatin replaces a C-5 carbonyl oxygen on HMG-CoA. This group hydrogen bonds with the carboxylate (or carboxyl) of Glu-559 in both E.I and enzyme–substrate complexes [5,12], and the C-5–OH of the inhibitor has a better matched pK$'_a$ than the C-5 = O of the HMG-CoA. However, the side chain of Glu-559 may function as a hydrogen-bond donor, because the pK$'_a$ is likely to be elevated above the usual value of around 4.5, due to the proximity of Asp-767 [12].

Another factor that may contribute to the affinity of rosuvastatin is that there are several groups on the inhibitor that form multiple hydrogen bonds with the enzyme. Hydrogen-bond formation in a complex usually gives a favourable shift in the enthalpy of binding and an entropic penalty. When a single group forms multiple hydrogen bonds, it is possible that the entropic penalty is smaller for the second and subsequent interactions. One of the carboxylate oxygens, the C-3–OH and the C-5–OH of rosuvastatin each form multiple hydrogen bonds with HMG-CoAR [5].

The increased affinity of rosuvastatin relative to that of the substrate also reflects the fact that the inhibitor makes more hydrophobic interactions with the enzyme [5]. These interactions are entropy driven. Binding of the inhibitor also appears to carry a smaller entropic penalty than binding of the substrate, because helix Lr11 is disordered in the complex with rosuvastatin, and ordered in the complex with HMG, CoA and NADP$^+$ [12].

Comparison of potency for different statins

Rosuvastatin is at least as potent as any of the other well-known statins (Table 1). It is clearly more potent than fluvastatin and pravastatin. The compound also exhibits over 1000-fold greater selectivity for inhibition of primary rat hepatocytes compared with cultured rat fibroblasts, due to active uptake into the liver-derived cells [7]. The compound is also relatively resistant to metabolism by human cytochrome P450 3A4. These characteristics probably contribute to its ability to reduce LDL cholesterol by up to 65% [7].

In conclusion, the mechanism, kinetics and thermodynamics of binding of rosuvastatin to HMG-CoAR have been determined. These properties, together with clinical evidence, indicate potential for the compound to be a highly effective new treatment for dyslipidaemia.

References


Received 18 December 2002