farms are vaccinated in ring vaccination. It is assumed that vaccination has no effect on previously infected farms.

12. The effective neighborhood size, n, in units of nearest neighbor farms, was estimated as

\[ n = \int_{0}^{R} \frac{g(r)}{2\pi r^2} dr \]

where R is given by the solution of

\[ \frac{g(r)}{2\pi r^2} = \frac{1}{r^2} \int_{0}^{r} g(r') \frac{dr'}{r'} \]

The connectedness of the contact network is given by

\[ \phi = \frac{1}{n^2} \int_{0}^{r} g(r') \frac{dr'}{r'} \]

where

\[ r = r' - r'' + r'' - 2z'\cos(\theta) \]


15. The population of farms was stratified into a susceptible class, S; sequential infection classes, I (1 = 1, 2, ...); and a slaughtered/vaccinated class, D. Multiple infected classes were used to exactly reproduce the gamma distribution fits to the delay data shown in Fig. 2 and to represent different stages of infectiousness and diagnosis. The mixture model of the infection-to-report distribution was represented by overlapping sets of 30 classes (transit time = 0.26 days each, weight 0.02) and 4 classes (transit times = 3.73 days, weight 0.18). Two classes (transit times = 0.85 to 0.21 days, time-dependent) represented farms awaiting disease confirmation after report, and four classes (transit times = 0.82 to 0.38 days, time-dependent)—overlapping the previous two—represented farms awaiting culling after disease reporting. Infectiousness varies as a function of incubation stage, reaching significant levels after around 3.5 days and then continuing at a constant level until diagnosis, after which it remains constant until slaughter, at a level r times greater than before reporting. The model is novel in tracking not only the numbers of farms in each infection state through time, but also the numbers of pairs of farms connected on the contact network used to represent spatially localized disease transmission. For conciseness and clarity, we only present those for a simpler model with only two infected classes: E (uninfected) and I (infectious). Using [X] to represent the mean number in state X, [XY] to represent the mean number of pairs of type XY, and [X][Y] to represent the mean number of triplets, the dynamics can be represented by the following set of differential equations:

\[
\frac{d[S]}{dt} = -[r + \mu + w]S + pt[S][N] + pt[E][N] - pt[S][N] - pt[E][N]
\]

\[
\frac{d[S]}{dt} = -[r + \mu + \omega]S - [\mu][S] - [\mu][N] - [\mu][E] - [\mu][S][N] - 2\mu[S][N]
\]

\[
\frac{d[E]}{dt} = -[r + \mu + \omega][E] + 2\mu[E][N] + [\mu][S][N] + [\mu][E][N] + [\mu][S][N] + [\mu][E][N]
\]

\[
\frac{d[A]}{dt} = -[r + \mu + \omega][A] + 2\mu[A][N] + [\mu][S][N] + [\mu][E][N] + [\mu][S][N] + [\mu][E][N]
\]

where A is the mean contact neighborhood size of a farm, \( \phi \) is the proportion of triplets in the network that are triangles, and N is the total number of farms [see (12)]. \( \tau = (1 - p)/\mu \) is the transmission rate across a contact, where p is the transmission coefficient of the virus, and \( \mu \) is the proportion of contacts that are long-range [see (9)], both of which are estimated separately before and after the movement back to baseline. The rate of transit from the infectious to the removed class is \( r \), the rate of transit from the infected to the removed class, \( \omega \) is the rate at which farms in the neighborhood of an infected farm are culled in ring culling, and \( \omega \) is the rate at which

Elevated cholesterol levels are a primary risk factor for coronary artery disease. This disease is a major problem in developed countries and currently affects 13 to 14 million adults in the United States alone. Dietary changes and drug therapy reduce serum cholesterol levels and dramatically decrease the risk of stroke and overall mortality (1). Inhibitors of HMGCR, commonly referred to as statins, are effective and safe drugs that are widely prescribed in the treatment of hypercholesterolemia. We have determined structures of the catalytic portion of human HMGCR complexed with six different statins. The statins occupy a portion of the binding site of HMG-CoA, thus blocking access of this substrate to the active site. Near the carboxyl terminus of HMGCR, several catalytically relevant residues are disordered in the enzyme–statin complexes. If these residues were not flexible, they would sterically hinder statin binding.

**Structural Mechanism for Statin Inhibition of HMG-CoA Reductase**

Eva S. István and Johann Deisenhofer

HMG-CoA (3-hydroxy-3-methylglutaryl–coenzyme A) reductase (HMGCR) catalyzes the committed step in cholesterol biosynthesis. Statins are HMGCR inhibitors with inhibition constant values in the nanomolar range that effectively lower serum cholesterol levels and are widely prescribed in the treatment of hypercholesterolemia. We have determined structures of the catalytic portion of human HMGCR complexed with six different statins. The statins occupy a portion of the binding site of HMG-CoA, thus blocking access of this substrate to the active site. Near the carboxyl terminus of HMGCR, several catalytically relevant residues are disordered in the enzyme–statin complexes. If these residues were not flexible, they would sterically hinder statin binding.

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HMG-CoA. (A) Structural formulas of statin inhibitors and the enzyme substrate of compactin (also known as mevastatin). We classify this group of inhibitors as type 1 statins. Fluvastatin, cerivastatin, atorvastatin, and rosuvastatin (in development by AstraZeneca) are fully synthetic HMGR inhibitors with larger groups linked to the HMGR-like moiety. We refer to these inhibitors as type 2 statins. The additional groups range in character from very hydrophobic (e.g., cerivastatin) to partly hydrophobic (e.g., rosuvastatin). All statins are competitive inhibitors of HMGR with respect to binding of the substrate HMG-CoA, but not with respect to binding of NADPH (6). The \( K_i \) (inhibition constant) values for the statin-enzyme complexes range between 0.1 to 2.3 nM (5), whereas the Michaelis constant, \( K_m \), for HMG-CoA is 4 \( \mu \)M (7).

Although the structure of the catalytic portion of human HMGR in complex with substrates and with products has recently been elucidated (8, 9), it yields little information concerning statin binding. The protein forms a tightly associated tetramer with bipartite active sites, in which neighboring monomers contribute residues to the active sites. The HMGR-binding pocket is characterized by a loop (residues 682–694, referred to as “cis loop”) (Fig. 2A). Because statins are competitive with respect to HMG-CoA, it appeared likely that their HMGR-like moieties might bind to the HMGR-binding portion of the enzyme active site. However, in this binding mode their bulky hydrophobic groups would clash with residues that compose the narrow pocket which accommodates the pantothenic acid moiety of CoA; thus, the mechanism of inhibition has remained unsolved.

To determine how statins prevent the binding of HMG-CoA, we solved six crystal structures of the catalytic portion of human HMGR bound to six different statin inhibitors at resolution limits of 2.3 Å or higher (Table 1) (10). For each structure, the bound inhibitors are well defined in the electron-density maps (Fig. 3). They extend into a narrow pocket where HMG is normally bound and are kinked at the OS-hydroxyl group of the HMGR-like moiety, which replaces the thioester oxygen atom found in the HMG-CoA substrate. The hydrophobic-ring structures of the statins contact residues within helices Lo1 and Lo10 of the enzyme’s large domain (Fig. 2B). No portion of the elongated NADPH (binding site is occupied by statins. The structures presented here illustrate that statins inhibit HMGR by binding to the active site of the enzyme, thus sterically preventing substrate from binding. This agrees well with kinetic studies that indicate that statins competitively inhibit HMG-CoA but do not affect NADPH binding (6).

A comparison between substrate-bound and inhibitor-bound HMGR structures clearly illustrates rearrangement of the substrate-binding pocket to accommodate statin molecules (Fig. 2). The structures differ in the COOH-terminal 28 amino acids of the protein. In the electron-density maps of the statin-complex structures, residues COOH-terminal to Gly860 are missing. In the substrate-complex structure, these residues encompass part of helix Lo10 and all of helix Lo11, fold over the substrate, and participate in the formation of the narrow pantothenic acid–binding pocket (Fig. 2A). In the statin-bound structures, these residues are disordered, revealing a shallow hydrophobic groove that accommodates the hydrophobic moieties of the statins.

![Fig. 2. Statins exploit the conformational flexibility of HMGR to create a hydrophobic binding pocket near the active site. (A) Active site of human HMGR in complex with HMG, CoA, and NADP. The active site is located at a monomer-monomer interface. One monomer is colored yellow, the other monomer is in blue. Selected side chains of residues that contact the substrates or the statin are shown in a ball-and-stick representation (20). Secondary structure elements are marked by black labels. HMG and CoA are colored in magenta; NADP is colored in green. To illustrate the molecular volume occupied by the substrates, transparent spheres with a radius of 1.6 Å are laid over the ball-and-stick representation of the substrates or the statin. (B) Binding of rosuvastatin to HMGR. Rosuvastatin is colored in purple; other colors and labels are as in (A). This figure and Figs. 3 and 4 were prepared with Bobscript (22), GLR (23), and POV-Ray (24).](www.sciencemag.org)
Although the structural changes in the complexes with statin had not been predicted, the COOH-terminal residues of HMGR are known to be a mobile element in this protein. In structures of the human enzyme in complex with HMG-CoA alone, helix Lα11 was partially disordered (8). Similarly, in structures of a bacterial homolog of HMGR from Pseudomonas mevalonii, a larger COOH-terminal domain that is not present in the human protein is disordered when no substrates are present (11) but ordered in the ternary complex (12). It appears that the innate flexibility of the COOH-terminal region of HMGR is fortuitously exploited by statins to create a binding site for the inhibitor molecules.

How is the specificity and tight binding of statin inhibitors achieved? The HMG-moieties of the statins occupy the enzyme active site of HMGR. The orientation and bonding interactions of the HMG moieties of the inhibitors clearly resemble those of the sub-

**Fig. 3.** Stereoview of the electron-density map of atorvastatin bound to the HMGR active site. This 2.2 Å simulated-annealing omit map, contoured at 1σ, was calculated by omitting all atoms of the atorvastatin molecule shown, as well as protein atoms within 4.5 Å of the inhibitor. The electron density is overlaid on the final, refined model. The electron density covering atorvastatin is in green, whereas the electron density covering the protein is in blue. Carbon atoms of one of the two protein monomers are colored yellow, those of the neighboring monomer are in blue, and those of atorvastatin are in gray. In all molecules oxygen atoms are red, nitrogen atoms are blue, sulfur atoms are yellow, and the fluorine atoms are green.

**Fig. 4.** Mode of binding of compactin (A), simvastatin (B), fluvastatin (C), cerivastatin (D), atorvastatin (E), and rosuvastatin (F) to human HMGR. Interactions between the HMG moieties of the statins and the protein are mostly ionic or polar. They are similar for all inhibitors and are indicated by the dotted lines. Numbers next to the lines indicate distances in Å (13). The rigid hydrophobic groups of the statins are situated in a shallow groove between helices Lα1 and Lα10. Additional interactions between Arg590 and the fluorophenyl group are present in the type 2 statins (C, D, E, F). Atorvastatin and rosuvastatin form a hydrogen bond between Ser565 and a carbonyl oxygen atom (atorvastatin) (E) or a sulfone oxygen atom (rosuvastatin) (F).
strate complex (Fig. 2). Several polar interactions are formed between the HMG-moieties and residues that are located in the cis loop (Ser658, Asp690, Lys691, Lys692). Lys691 also participates in a hydrogen-bonding network with Glu239, Asp378 and the O5-hydroxyl of the statins. The terminal carboxylate of the HMG moiety forms a salt bridge to Lys737. The large number of hydrogen bonds and ion pairs results in charge and shape complementarity between the protein and the HMG-like moiety of the statins. Identical bonding interactions are observed between the protein and HMG and presumably also with the reaction product mevalonate (Fig. 2A). Because mevalonate is released from the active site, it is likely that not all of its interactions with the protein are stabilizing. These observations suggest that the hydrophobic groups of the inhibitors are predominately responsible for the nanomolar $K_i$ values; they may also change the context of the HMG-like polar interactions such that the ion pairs contribute favorably to the binding of statins.

Hydrophobic side chains of the enzyme involving residues Leu656, Val657, Leu658, Ala659, and Leu677 participate in van der Waals contacts with the statins. The surface complementarity between HMGR and the hydrophobic ring structures of the statins is present in all enzyme-inhibitor complexes, despite the structural diversity of these compounds. This is possible because the type 1 and type 2 statins adopt different conformations that allow their hydrophobic groups to maximize contacts with the hydrophobic pocket on the protein (Fig. 4). Functionally, the methylethyl group attached to the central ring of the type 2 statins replaces the decalin of the type 1 statins. The butyryl group of the type 1 statins occupies a region similar to the fluorophenyl group present in the type 2 inhibitors.

A comparison between the six complex structures illustrates subtle differences in their modes of binding. Rosuvastatin has the greatest number of bonding interactions with HMGR (Fig. 4F). In addition to numerous contacts present in other statin-HMGR complex structures, a polar interaction between the Arg568 side chain and the electronegative sulfone group is unique to rosuvastatin. Present only in atorvastatin and rosuvastatin are hydrogen bonds between Ser658 and either a carbonyl oxygen atom (atorvastatin) or a sulfone oxygen atom (rosuvastatin) (Fig. 4, E and F). The fluorophenyl groups of type 2 statins are one of the main features distinguishing type 2 from the type 1 statins. Here, the guanidinium group of Arg590 stacks on the fluorophenyl group, and polar interactions between the arginine $n$ nitrogen atoms and the fluorine atoms are observed. No differences between the type 1 statins compactin and simvastatin are apparent (Fig. 4, A and B). With the exception of the larger atorvastatin, the solvent-accessible areas of unbound or bound statins and the buried areas upon statin binding to HMGR are similar for all inhibitors (13).

In summary, these studies reveal how statins bind to and inhibit their target, human HMGR. The bulky, hydrophobic compounds of statins occupy the HMG-binding pocket and part of the binding surface for CoA. Thus, access of the substrate HMG-CoA to HMGR is blocked when statins are bound. The tight binding of statins is probably due to the large number of van der Waals interactions between inhibitors and with HMGR. The structurally diverse, rigid hydrophobic groups of the statins are accommodated in a shallow non-polar groove that is present only when COOH-terminal residues of HMGR are disordered. Although the statins that are currently available or in late-stage development excel in curtailing the biosynthesis of mevalonate, the precursor of cholesterol, it is possible that the visualization of statin bound to HMGR will assist in the development of even better inhibitors. In particular, it should be noted that the nicotinamid-bound site of HMGR is not occupied by statin inhibitors and that the covalent attachment of a nicotinamide-like moiety to statins might improve their potency.

References and Notes

10. The catalytic portion of human HMGR was purified as described (8). Concentrated stock solutions of the inhibitors were prepared in methanol and added to the protein in three- or fourfold molar excess. Simvastatin, fluvastatin, cerivastatin, atorvastatin, and rosuvastatin were received from AstraZeneca and were in their active hydroxy-acid form. Compaction was purchased from Sigma and activated by converting the lactone form to the sodium salt with NaOH as described (14). After a 6 to 24 hour incubation of protein with inhibitor at 4°C, batch crystallization trials at 21°C were set up. Crystals were grown at a protein concentration of 3 to 5 mg/ml and in solutions containing 12 to 15 % [weight/volume (w/v)] polyethylene glycol (PEG) 4000, 0.15 to 0.2 M ammonium acetate, 25 mM Na-Hepes (pH 7.5), 50 mM HEPES.
Control of a Genetic Regulatory Network by a Selector Gene

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The formation of many complex structures is controlled by a special class of transcription factors encoded by selector genes. It is shown that SCALLOPED, the DNA binding component of the selector protein complex for the Drosophila wing field, binds to and directly regulates the cis-regulatory elements of many individual target genes within the genetic regulatory network controlling wing development. Furthermore, combinations of binding sites for SCALLOPED and transcriptional effectors of signaling pathways are necessary and sufficient to specify wing-specific responses to different signaling pathways. The obligate integration of selector and signaling protein inputs on cis-regulatory DNA may be a general mechanism by which selector proteins control extensive genetic regulatory networks during development.

The concept of the morphogenetic field, a discrete set of cells in the embryo that gives rise to a particular structure, has held great importance in experimental embryology (1). The discovery of genes whose products control the formation and identity of various fields, dubbed "selector genes" (2), has enabled the recognition and understanding of discrete territories of selector gene activity (3). Although the term has been used somewhat liberally, two kinds of selector genes have been of central interest: SD is a TEA-domain protein (9) that binds to DNA in a sequence-specific manner (7), whereas VG, a novel nuclear protein (10), functions as a trans-activator (11). To determine whether direct regulation by SD is widely required for gene expression in the wing field, we analyzed the regulation of several genes that represent different nodes in the wing genetic regulatory network and that control the development of different wing pattern elements (Fig. 1A). We focused in particular on genes for which cis-regulatory elements that control expression in the wing imaginal disc have been isolated, including cut (12), split (sal) (13), and vg (6).

We first tested whether sd gene function was required for the expression of various genes in the wing field. We generated mitotic clones of cells homozygous for a strong hypomorphic allele of sal and assessed the expression of gene products or reporter genes within these clones (14). Reduction of sd function led to a diminution of the expression of the CUT (Fig. 1, B and F) and WINGLESS (WG) (Fig. 1, C and G) proteins and of reporter genes under the control of the sal 10.2-kb (Fig. 1, D and H) and the vg quadrant (Fig. 1, E and I) enhancers.