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Structure, dynamics and selectivity in the sulfotransferase family

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Abstract

Combined structure, function and molecular dynamics studies of human cytosolic sulfotransferases (SULT1A1 and 2A1) have revealed that these enzymes contain a ~30-residue active-site cap whose structure responds to substrates and mediates their interactions. The binding of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) gates access to the active site by a remodeling of the cap that constricts the pore through which acceptors must pass to enter the active site. While the PAPS-bound enzyme spends the majority (~95%) of its time in the constricted state, the pore isomerizes between the open and closed states when the nucleotide (PAPS) is bound. The dimensions of the open and closed pores place widely different steric constraints on substrate selectivity. Nature appears to have crafted these enzymes with two specificity settings – a closed-pore setting that admits a set of closely related structures, and an open setting that allows a far wider spectrum of acceptor geometries. The specificities of these settings seem well matched to the metabolic demands for homeostatic and defensive SULT functions. The departure of nucleotide requires that the cap open. This isomerization dependent release can explain both the product bursts and substrate inhibition seen in many SULTs. Here, the experimental underpinnings of the cap-mechanism are reviewed, and the advantages of such a mechanism are considered in the context of the cellular and metabolic environment in which these enzymes operate.

Keywords

Isomerization; metabolism; molecular dynamics; structure; substrate inhibition; sulfotransferase; SULT; synergy

The human cytosolic sulfotransferases (SULTs) comprise a 13-member family of enzymes that catalyze transfer of the sulfonyl-group ($-\text{SO}_3$) from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the hydroxyl and primary amine moieties of thousands of endogenous and xenobiotic acceptors. Receipt of the sulfonyl-group typically weakens (Allali-Hassani et al., 2007; Kotov et al., 1999), but sometimes strengthens (Cook et al., 2009) ligand/target-interactions, often by several orders of magnitude. SULTs sulfonate numerous receptor agonists and antagonists and are thus crucial to signaling homeostasis.

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Declaration of interest

The authors report no conflicts of interest. They alone are responsible for the content and writing of this work.

Given the breadth and depth of its metabolic involvement, it is perhaps not surprising that malfunctioning sulfuryl-transfer leads to disease including cancers of the breast (Falany et al., 2002) and endometrium (Falany & Falany, 1996), cholestasis (Zollner & Trauner, 2006), metachromatic leukodystrophy (Poeppel et al., 2005), hemophilia (Moore, 2003) and atherosclerosis (Bai et al., 2011; Kase et al., 2006).

Understanding how SULTs select particular substrates from a complex cytosolic *milieu* that contains scores of competing ligands is fundamental to understanding their functions in biology. Recent biophysical studies have revealed that selectivity in binding and catalysis relies in large measure on the plasticity of a conserved active-site cap that mediates the substrate interactions that determine their affinities and the rates at which they enter and depart from the active site.

The SULT active-site cap is a dynamic ~30 residue stretch of amino acids that interacts with and encapsulates both the donor and acceptor substrates (Cook et al., 2012). Structures of nucleotide-bound SULTs show a cap that is largely ordered regardless of whether acceptor is bound (Dong et al., 2012; Pedersen et al., 2000, 2002). In contrast, the caps seen in structures that contain only acceptor are highly disordered (Chang et al., 2004; Lu et al., 2008; Rehse et al., 2002). These findings suggested that the cap is dynamic, that it responds to ligand, and that it likely plays an important role in substrate recognition (Cook et al., 2013a).

To investigate possible roles of the cap in substrate binding, molecular dynamics models of the nucleotide-bound and ligand-free structures of SULT2A1 were constructed (Figure 1) (Cook et al., 2013a). The predicted PAPS-bound, or closed, structure closely resembles that seen in SULT crystal structures (Pedersen et al., 2000), and the disordered cap predicted for the ligand-free, or open, enzyme is consistent with the fact that these residues are not resolved in the structural data (Chang et al., 2004). The modeled structures are presented in Figure 1 in a tubular format for which the root mean square fluctuations (RMSF) of the α -carbons are given by the color and width of the tube, which are calibrated in the inset scale. In the E · PAPS structure (lower left), the cap strand interfaces with two complementary strands to form a pore whose center is given by a red sphere, which also marks the approximate active-site point-of-entry of acceptors. Each of the three strands is highlighted by a transparent grey surface. Upon loss of nucleotide, the cap dislodges from the other strands and becomes highly disordered and dynamic (C_{α} -RMSF values range as high as 3.7 Å). The transparent grey surfaces in the open structure provide a visual cue for the changes that occur upon loss of nucleotide. As can be seen, the active-site pore undergoes a considerable expansion as the system opens. Consistent with structural data, these simulations suggest that nucleotide binding gates access to the active site by controlling the status of the pore (Cook et al., 2013a).

If, in fact, nucleotide gates access to the acceptor-binding pocket, ligands whose dimensions exceed those of the closed pore will compete with nucleotide for the open enzyme, and the affinity of either ligand will appear to weaken in the presence of other (Cook et al., 2012). In contrast, active site access to ligands small enough to pass through the closed pore should not be restricted by the position of the gate (or the presence of nucleotide). The two ligands

selected to test the gating mechanism are dehydroepiandrosterone (DHEA) and raloxifene. DHEA is a classic substrate for SULT2A1, also known as DHEA SULT (Falany et al., 1989). DHEA is the most abundant steroid in plasma (Mesiano & Jaffe, 1997). It circulates as DHEA-sulfate and is converted *in-situ* to any number of other steroids (Labrie et al., 1998). Raloxifene (Evista®), one of many drugs sulfated by SULT2A1 (Falany et al., 2006), is taken daily by approximately 1.2 million women in the United States to prevent osteoporosis and decrease the risk of breast cancer (Jordan, 2007). Raloxifene is composed of a steroid-like “base” whose dimensions are comparable to those of DHEA, and a large R-group whose dimensions are also similar to DHEA (Figure 2). To assess their ability to bind to the open and closed forms of the enzyme, DHEA and raloxifene were docked into these structures using GOLD (Cook et al., 2012; Verdonk et al., 2004) (panels A and B, Figure 3). The edge of the cap that forms the gate is shown in blue (open) and white (closed). The models predict that DHEA positions well for catalysis in either the open or closed structures, and that raloxifene is sterically prevented from accessing the closed active site.

SULTs are rich in fluorescent amino acids, which are often concentrated at or near the active site. These enzymes typically undergo significant intrinsic fluorescence changes that can be used to assess the binding and interactions of donors and acceptors (Cook et al., 2012; Sun & Leyh, 2010; Zhang et al., 1998). Because it is non-reactive, PAP is often used in place of PAPS in equilibrium-binding studies. PAP is an excellent PAPS surrogate. Their affinities and acceptor interactions are identical within error (Cook et al., 2012; Zhang et al., 1998). The binding of DHEA to SULT2A1 in the presence and absence of PAP is shown in Figure 4(A) (Cook et al., 2012). The affinities of DHEA for PAP and E · PAP are virtually identical (1.0 ± 0.08 and 1.1 ± 0.2 μ M, respectively). On the other hand, K_d (raloxifene) increases 21-fold (1.1 ± 0.2 to 29 ± 4 μ M) at a saturating concentration of PAP (Cook et al., 2012), Figure 4(B). Similar findings were obtained in initial-rate studies using DHEA or raloxifene – K_m and K_i for DHEA are nearly identical; whereas, K_m is 23-fold greater than K_i for raloxifene (Cook et al., 2012). These results provided the first clear examples of antisynnergistic interactions between substrates in the SULT family.

The gating model predicts that the affinity of raloxifene will decrease in the presence of PAP and *vice versa* – which is observed (Cook et al., 2012). However, it also predicts that these ligands compete for the open enzyme. If this were true, the affinity of a ligand like raloxifene would tend toward zero as the concentration of PAPS approached infinity, which is not the case. Furthermore, if binding of these ligands were mutually exclusive, turnover, which requires that both ligands be present on the enzyme simultaneously, would not occur. Thus, the simple competitive model cannot accommodate the data.

The discrepancy between the competitive model and the data is reconciled if the model allows the enzyme to isomerize between open and closed states when nucleotide is bound (Cook et al., 2012, 2013a). The question of whether such an isomerization occurs was answered by studying the rate at which ligands bind using presteady state fluorescence (Cook et al., 2012). Ligand binding is a second-order process whose rate is given by the product of the concentration of ligand, enzyme and the on-rate constant ($v = k_{on} [A] [E]$). If nucleotide shifts the enzyme into the closed state, the concentration of the open form decreases, and the rate at which compounds like raloxifene, which bind only the open form,

will decrease commensurately. However, departure of the ligand is a first-order process, and is thus independent of enzyme concentration. In short, the nucleotide induced changes in the affinity of large substrates should appear to be exclusively on the on-rate constant. In contrast, neither the on-rate nor the off-rate constant of compounds like DHEA, which are small enough to bind to the closed form, will be affected by the position of the gate. These predictions were supported by experimentation that demonstrated that bound nucleotide decreases the rate of raloxifene binding 22-fold without affecting the rate at which it departs from the enzyme, and has no effect on the ad- or desorption of DHEA (Cook et al., 2012).

Ligand binding studies confirm that a nucleotide induced isomerization limits active-site access to large acceptors (Cook et al., 2012, 2013a) and crystal structures offer a plausible framework for the isomerization (Cook et al., 2013b; Pedersen et al., 2000; Rehse et al., 2002). To test that framework, amino acid R-groups predicted to stabilize the closed cap were replaced, *via* mutagenesis, with R-groups expected to weaken the interactions and destabilize the closed cap (Cook et al., 2013b). If the structural basis of the isomerization is in fact cap closure, such mutations will shift the isomerization equilibrium constant toward the open state and thus minimize the effects of nucleotide on acceptor binding. The stabilizing amino acids and their replacements were assessed in *in-silico* studies prior to experiment (Cook et al., 2013a). The resulting target residues are numbered and color coded in Figure 5. Targets were selected from several different regions of the cap to ensure a robust, multi-point test of the cap model.

Mutations at any of the three target sites produced similar results – nucleotide binding is completely uncoupled from the binding of large acceptors (Cook et al., 2013b). That is, the affinities of large acceptors for the E and E · PAP forms of the mutant enzymes are identical. Large-substrate affinities for the E forms were the same for the wild-type and mutant enzymes; hence, the mutations impaired isomerization without altering the intrinsic potential of the binding pocket. Furthermore, the fact that the initial-rate parameters of small substrates are not affected by the mutations indicates that they do not significantly perturb the catalytic “machinery.” It is notable that for large substrates, cap destabilizing mutations both decrease the K_m (18-fold) and increase k_{cat} (10-fold). These mutants increase catalytic efficiency (k_{cat}/K_m) 180-fold (Cook et al., 2013b). Given that SULT turnover is product release rate limited (Sun & Leyh, 2010; Tyapochkin et al., 2008; Zhang et al., 1998), these findings suggest a correlation between cap destabilization and the rate of product release (Cook et al., 2013b).

Further insight into the structural basis of coupling between nucleotide binding and cap closure was obtained in a combined crystallographic and computational study of SULT2A1 (Cook et al., 2013a,b). Weakening the interactions between the cap and base of the active site *in-silico* causes the cap to open and close in “halves.” One half covers the donor; the other, the acceptor (Cook et al., 2013a). Weakening these same interactions experimentally, *via* mutagenesis, decreases the energetics of the donor/acceptor interactions to near zero (Cook et al., 2013b). The half-cap, or segmental motion model was confirmed when the structure of the double mutant (LL233/234GG, lime-green cap residues, Figure 5) bound to PAP was solved (Cook et al., 2013b). The structure of the half-open cap is superposed on the wild-type cap in Figure 6. The mutant cap is presented in a transparent putty whose color

and diameter scale with the mobility of the C α atoms. The wild-type cap is in blue and rendered in cartoon. Remarkably, the structure of the E · PAP complex shows the acceptor half of the mutated cap detached from the base and opened at precisely the molecular “hinge-points” predicted by the simulations (Cook et al., 2013a). The fact that mutations at any of the three positions shown in Figure 6 leads to the same outcome – an uncoupling of the binding of the donor and acceptor – reveals that weakening the cap at any of a number of points leads to a concerted detachment of a set of contiguous residues – the half-cap (Cook et al., 2013b). Given their proximity, it is perhaps not surprising that these residues attach and detach in a synergistic fashion.

Gated product release and substrate inhibition

Partial substrate inhibition is commonly observed in the SULT family. The diagnostic pattern for this type of inhibition is shown in Figure 7 (Sun & Leyh, 2010) in which the initial-rate of reaction is plotted versus substrate concentration, [S]. As [S] increases, the rate increases and then decreases to a plateau. The fact that the rate plateaus at a non-zero value identifies the inhibition as “partial.” The shape of the inhibition region of the curve is consistent with a simple titration of the enzyme into a form in which steady-state turnover is slowed. Despite its apparent simplicity, the mechanistic underpinnings of this curve-shape have been debated for more than three decades (Duffel & Jakoby, 1981). Explanations include subunit interactions (Petrotchenko et al., 2001), non-productive binding, allosteric binding (Lu et al., 2008), multiple active-site substrates (Gamage et al., 2003) and dead-end complex formation (Gulcan & Duffel, 2011; Sun & Leyh, 2010; Tyapochkin et al., 2009). Here we consider for the first time, that gating of the cap is fundamental to substrate inhibition and highlight recent findings that support this concept.

Structures suggest that nucleotide is encapsulated by the closed active site to an extent that it cannot depart without opening the cap (Allali-Hassani et al., 2007; Dong et al., 2012; Gamage et al., 2005; Pedersen et al., 2000). The nucleotide is shown encapsulated in the E · PAP complex of SULT2A1 in Figure 8 (Pedersen et al., 2000). As required by conservation of energy, nucleotide and large substrates affect one another’s affinity equally. For example, the affinities of raloxifene and PAPS for SULTA1 each decrease 22-fold when the other ligand is bound. If nucleotide departs only from the cap-open form of the enzyme, the rate constant for its departure will increase 22-fold when a large substrate, like raloxifene, is bound. This precisely what is observed (Cook et al., 2012), thus gating controls the release of the nucleotide.

Given that nucleotide release is gated by cap fluctuations, one can ask: “How might cap-mediated nucleotide release be related to substrate inhibition?” Thus far, three SULTs have been shown to produce a burst of product in the presteady state phase of the forward (PAP-forming) reaction (Cook et al., 2010; Sun & Leyh, 2010; Tyapochkin et al., 2008). The burst amplitude reveals that one active-site equivalent of product is formed per dimer, and detailed analysis reveals that these SULTs are so-called half-site enzymes; that is, only one of the two active sites turns over per catalytic cycle (Sun & Leyh, 2010). Product bursts are indicative of, but not exclusive to, rate-limiting product release (Sun & Leyh, 2010). Recent work has shown that substrate concentrations that inhibit steady-state turnover do not affect the

amplitude or rate of formation of the burst (Sun & Leyh, 2010). Hence, the steps that are slowed in achieving inhibition must occur after formation of the product central complex (i.e. $E \cdot PAP \cdot AS$, where AS is the sulfonated acceptor). The formation of dead-end ternary complexes containing PAP and acceptor is well established (Gulcan & Duffel, 2011; Kakuta et al., 1997; Sun & Leyh, 2010; Zhang et al., 1998). Thus it is plausible that substrate inhibition is due to the formation of a ternary complex that decreases the rate of PAP release by decreasing the fraction of the enzyme in the cap-open form. Consistent with this model, DHEA slows the release of PAPS from SULT2A1 by ~20% and tightens PAP binding by 30% (Cook et al., 2012). The similarity between these numbers and the ~30% decrease in k_{cat} caused by DHEA inhibition (Falany et al., 1989) lends credence to the mechanism; however, its validation requires further experimentation.

Does substrate inhibition occur *in vivo*?

While substrate inhibition is common among SULTs, the inhibition constants are often high enough ($\sim 10 \cdot K_m$) to call into question whether substrate inhibition is significant *in vivo* (Mesiano & Jaffe, 1997; Mishell et al., 1973; Morris & Levy, 1983). It is important here to consider that because of their broad specificity, SULTs are inhibited by many substrates (Berger et al., 2011; Lu et al., 2008, 2010). Consequently, it is the collective action of these substrates that determines the fraction of the enzyme that is inhibited. Because chemical potentials are additive, the thermodynamic driving force of a single substrate at $10 \cdot K_d$ is equivalent to that of ten substrates each at $1 \cdot K_d$. Either scenario produces the same fraction of bound enzyme. This concept is illustrated in Figure 9, which shows the inhibition of a substrate-inhibited enzyme as a function of an increasing number of substrates, each of which is at its K_m . The inhibition constant in the simulation, K_i , was set at $10 \cdot K_m$. If a cell contained 50 substrates, each at its K_m , the collective concentration would be $5 \cdot K_i$, which is the equivalent of one substrate at $5 \cdot K_i$ – 83% of the enzyme is inhibited in either case. We are not aware of studies that characterize the numbers and levels of SULT substrates in cellular cytosols; however, given the many hundreds of compounds that are sulfated by SULTs in hepatocytes it would not be surprising to find numerous substrates in the cytosol at any given moment (Allali-Hassani et al., 2007; Falany, 1997).

The metabolic utility of dual specificity

SULTs have two specificity “settings” – broad and narrow – and they distribute between these settings according to the isomerization equilibrium constant, K_{iso} . At saturating nucleotide and in the absence of acceptor, a single enzyme molecule will spend on average ~5% of its time in the broad-specificity or open state (Cook et al., 2013a). These facts beg the following questions: “What is the value of dual specificity in the SULT family, and why bias specificity strongly toward the narrow setting?”

The acceptor specificity of the closed form of a given SULT centers on the metabolites on which that SULT operates. Together, this structurally related set of compounds provides a composite geometric volume whose outer surface roughly defines the limits of the compounds that can pass through the closed pore. Compounds whose dimensions lie within these limits have unrestricted access to the active site, and their catalytic efficiencies and

affinities are not influenced by the state of the pore. Substrates whose structures exceed the limits cannot pass through the closed pore and must pay an energetic price for binding that is given by the energy needed to open the pore. Closure of the pore causes the affinities of large substrates to decrease by a factor of $1/K_{\text{iso}}$. Thus, isomerization “protects” homeostatic functions by thermodynamically favoring the closed form, yet it does so in a way that allows the enzyme to remain responsive to large substrates. At high enough large-substrate concentrations, the enzyme can be entirely engaged in large substrate catalysis. This two-stage system provides an on-demand reservoir of catalytic power that can be titrated into action by an increase in the cumulative concentration of large substrates.

The affinities of large and small substrates for the open form of the enzyme are comparable, and small-substrate affinities are not influenced by the position of the cap. Thus, the binding determinants that interact with the steroid-like base of large and small substrates operate largely independently of the cap, and it is solely the stability of the cap, which is given by K_{iso} , that determines the factor by which large and small substrate affinities differ. One cannot help but wonder whether K_{iso} has been “tuned” by evolutionary forces to achieve a K_{m} that is “set” near metabolite concentrations (Gillespie, 1981). During digestion, thousands of xenobiotics enter the liver and their concentrations in hepatocytes are expected to increase markedly (Latté et al., 2011; Shore et al., 1993; Waterhouse, 2002). To the extent that K_{m} can be used as a measure of these levels, one can expect aggregate levels of large substrates to rise to $\sim 20 \mu\text{M}$ during peak intervals.

The design of the two-state, steric screen

The open and closed forms of the pore parse substrates into two groups, large and small. This selection appears to be purely steric (Cook et al., 2012, 2013a) and may seem somewhat crude in its design in that any molecules small enough to enter the pore can access the active site. It is important to recognize that this coarse-grained screen is among the earliest steps (perhaps the first) in an overall process that includes multiple selective interactions, each of which enhances or diminishes the affinity of the substrate by a factor equal to the equilibrium constant for that interaction.

The set of xenobiotic substrates that must be handled by SULTs is enormous (Falany, 1997; Nowell & Falany, 2006) and is a sensitive function of the environment, diet and metabolism of the individual. Meals present substrate sets that can be as different as the molecular contents of broccoli and steak (Latté et al., 2011; Van Koevering et al., 1995). While no two individuals are identical, all individuals must be served by a tiny, evolutionarily stable set of SULTs. Given this task, it is perhaps not surprising that nature has designed SULTs to begin substrate selection with a rough separation into xenobiotic and endogenous compounds based on their geometries.

SULT behavior at cytosolic concentration

Conditions in the hepatocyte cytosol are not well understood. We do know that the protein concentration in cytosol is quite high ($\sim 300 \text{ mg/ml}$) (Falany et al., 1990; Renwick et al., 2002; Wynne et al., 1992) and that the concentrations of SULTs 1A1 and 2A1 are 0.3% and

0.15% of that value, respectively (Riches et al., 2009; Teubner et al., 2007). These numbers predict that the active-site concentration of SULTs 1A1 and 2A1 are ~24 and 12 μM , respectively. Given that subcellular organelles, which exclude SULTs (Falany et al., 1998), comprise ~40% of the cytosol volume (Goresky, 1963; Hallbrucker et al., 1991; Okanoue et al., 1985), the SULT concentrations are estimated to increase to 40 and 20 μM . These concentrations are far higher than typical acceptor affinity constants, ~1 μM . At these concentrations it is perhaps better to consider that acceptors in the cytosol are saturated with enzyme, rather than the converse. Notably, the sum of these active-site concentrations, ~60 μM , is slightly less than the estimates of PAPS concentration in hepatocytes, ~80 μM (Alnouti & Klaassen, 2006; Klaassen & Boles, 1997). Under such conditions, the majority of PAPS is enzyme bound and the enzymes are saturated with nucleotide and fully biased toward the closed conformation. At this concentration, SULTs have the capacity to “saturate” an aggregate ~50 μM of acceptors. At concentrations at or below this value, the majority of small acceptors are bound and thus do not compete for the enzyme. Consequently, metabolites need not enter competitive queues that would otherwise delay their sulfonation. While plausible, confirmation of this theory will require a determination of the aggregate concentration of acceptor in the hepatocyte cytosol – an topic about which very little is known.

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Figure 1.

The open and closed forms of SULT2A1 *in silico*. The predicted structures and α -carbon dynamics of SULT2A1 are shown with and without bound PAPS. The small red sphere marks the entrance to the acceptor binding pocket which is formed by the three segments of the E · PAPS structure that are highlighted by transparent surfaces. The dynamics (root-mean-squared-fluctuations, RMSF) of the α -carbon backbone are given by the width and color of the chain. As a visual aid, the transparent surfaces seen in the closed state (E · PAPS) are superposed onto the open (unliganded) structure.

Figure 2.

The structures of DHEA and raloxifene. DHEA (3S,8R, 9S,10R,13S,14S)-3-hydroxy-10,13-dimethyl-1,2,3,4,7,8,9,11,12,14,15, 16-dodecahydrocyclopenta[a]phenanthren-17-one), and raloxifene [6-hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl]-[4-(2-piperidin-1-ylethoxy)phenyl]methanone.

Figure 3. Nucleotide-linked gate closure discriminates substrates. (A) DHEA is well positioned to engage in chemistry in either the open or closed complexes. (B) Raloxifene cannot access its binding pocket when the gate is closed (white), but can bind and react when it is open (blue). Ligands are positioned based on *in-silico* docking studies (see text).

Figure 4.

Equilibrium binding of RAL and DHEA to SULT2A1. (A) DHEA binding to E and E · PAP. Binding is monitored by changes in intrinsic enzyme fluorescence ($\lambda_{\text{ex}}=290$, $\lambda_{\text{em}}=340$). Solution composition and conditions: SULT2A1 (0.20 μM), PAP (0 or 125 μM ($600 \times K_d$)), MgCl_2 (5.0 mM), KPO_4 (25 mM, pH 7.4), 25 ± 2 °C. Fluorescence intensity (I) is normalized to total fluorescence change (I_{total}). Lines through the data indicate the least-squares best-fit using a model that assumes a single binding site *per* subunit. (B) Raloxifene

binding to E and E · PAP. Conditions were identical to those described in Panel A except that PAP was 25 μ M ($4.2 \times K_d$).

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Figure 5.
Cap stabilizing interactions selected for mutagenesis. The closed SULT2A1 · PAP configuration is shown. Each of the interactions is “broken” as the cap separates from the base. Removing these interactions *via* mutagenesis causes the cap to open and uncouples the linkage between nucleotide and acceptor binding.

Figure 6.

The active site caps of wild-type and mutant SULT2A1. Structures of the E · PAPS complex of wild-type and double-mutant (LL233/234GG) SULT2A1 are superposed. The wild-type (WT) cap is rendered in blue-grey cartoon, the mutant (MT) cap is shown in semi-transparent B-factor putty whose color and width scale with C_α B-factor. Acceptor (DHEA) was docked into the WT structure to provide a visual cue for substrate positioning. Red spheres identify the C_α atoms predicted by MD models to act as “hinges” for opening and closure of the acceptor half of the binding pocket. The MT cap indeed opens at the hinges and is disordered throughout its acceptor segment (residues 232–241); the WT cap is disordered only at positions 240–241.

Figure 7.

Partial substrate inhibition of SULT1E1 by estradiol. The v versus $[S]$ curve is defined by two phases. At low $[S]$ the initial rate increases with increasing $[S]$. At higher concentrations, the initial rate decreases as the enzyme is titrated into a form(s) with a slowed turnover. The non-zero plateau defines the inhibition as “partial”.

Figure 8.

The closed structure of SULT2A1 encapsulates PAPS. The active-site surface of the closed form of SULT2A1 is shown “wrapped” around the nucleotide. The limited access of nucleotide to solvent is highlighted by the dashed line that circumscribes the only solvent accessible surface of the nucleotide. Structural change appears to be required for nucleotide release.

Figure 9.

Modeling inhibition with multiple substrates. The percent of enzyme in the inhibited complex is shown as a function of the number of substrates. The concentration of each substrate is equal to its K_d . K_i is set at $10 \cdot K_d$. For SULT1A1 and 2A1, K_d and K_m are identical within error.