Modeling the Binding and Inhibition Mechanism of Nucleotides and Sulfotransferase Using Molecular Docking

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Phenol sulfotransferase (PST) catalyzes sulfuryl group transfer from adenosine 3′-phosphate 5′-phosphosulfate (PAPS) to a variety of nucleophilic acceptors in biological systems. Physiological sulfation by PAPS results in the production of adenosine 3′,5′-diphosphate (PAP). PAP may become a strong inhibitor or cofactor for the physiological or the transfer reactions, respectively. Several nucleotides other than PAP also serve as substrates, inhibitors or cofactors of PST catalyzed reactions. We are interested in the effects of these nucleotides on the PST catalyzed reactions and their possible physiological significance in biology. Several nucleotide inhibitors (such as adenosine 5′-diphosphate and adenosine 5′-triphosphate) of sulfotransferase in physiological reactions have been reported in the literature. However, our data suggests that they are not inhibitors for the transfer reaction or the reverse physiological reaction catalyzed by PST. The aim of this work is to show that the molecular docking analysis can be successfully used to underline the inhibition mechanism of these nucleotides. First, the selected compounds were subjected to a detailed docking analysis, by means of GEMDOCK, a program able to reveal the most likely binding mode for each ligand. By comparing these results with binding sites and binding compounds of the nucleotides with known X-ray structures, it is possible to highlight the site specificity and the inhibition mechanism of the select compounds. The results obtained by the above algorithm were further confirmed experimentally. In this paper, we show the effect of a variety of nucleotides on the activity of sulfotransferase in different docking conditions.

Keywords: Phenol sulfotransferase; 3′-Phosphoadenosine 5′-phosphosulfate; Adenosine 3′,5′-bisphosphate; Nucleotide.

INTRODUCTION

Recent advances in protein structure determination, via nuclear magnetic resonance, X-ray crystallography, or computer modeling, are providing the necessary data for biochemists, chemists, and pharmacologists to design and study ligands/substrates for these proteins. Identifying the ligands that bind in the active site of these newly determined protein structures has led to the development of a variety of docking strategies.[1] In the case of sulfotransferase, starting with a known active site and a database of compounds, it would be advantageous to be able to quickly identify a few compounds that "dock" into the active site and calculate "good" binding free energies. There are various interactions between a substrate and protein that must be modeled. Such interactions include shape complementarity, interaction specificity (charge-charge interactions), as well as solvation/desolvation interactions, hydrophobic interactions, and hydrogen bonding.[2,3] With the inclusion of each interaction, substrate-protein complexes are better predicted.

It has been known for a long time that sulfation occurs in a biological system.[4,5] Compounds that involve sulfation and the hydrolysis of sulfate esters include drugs, carcinogens, other xenobiotics,[6,7] hormones,[8] bile acids,[9] neurotransmitters,[10] glycoproteins, glycosaminoglycans, and saccharides.[11] Different isoforms of sulfotransferases have been isolated from mammals, bacteria, and plants.[12] Unlike a phosphorylation reaction,[13] the mechanism of an enzymatic sulfuryl group transfer has yet to be understood.[14-16] However, the resolution of crystal structures of the sulfotransferases

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family[17-23] produces important advances for our understanding in the reaction of these enzymes.

A sulfated nucleotide, 3′-phospho adenosine 5′-phosphosulfate (PAPS), is used for physiological sulfation which is catalyzed by sulfotransferases (EC 2.8.2). As shown in reaction 1, sulfotransferases are responsible for all the known sulfuryl group transfer reactions.[6-11] As shown in Reaction 2, sulfotransferase with the catalytic amount of adenosine 3′,5′-bisphosphate (PAP) can carry out a two-step sulfuryl group transfer.[14,24-26] Phenol sulfotransferase catalyzes both reactions,[24] with the activity in the physiological direction (Reaction 1) almost 10-fold slower than that in the transfer reaction (Reaction 2). The binding of PAP[27] and oxidation/reduction of cysteine[28-30] (which in turn affects the PAP binding) may be the reason for the rate difference of these two reactions.

$$\text{PAPS} + \text{HO-R} \rightarrow \text{PAP} + \text{R-OSO}_3^{2-} \quad (1)$$

$$\text{R-OSO}_3^{2-} + \text{R'-OH} \rightarrow \text{R-OH} + \text{R'-OSO}_3^{2-} \quad (2)$$

The sulfation of a nucleotide is an indispensable step for the sulfuryl group transfers in a biological system. The product and cosubstrate of sulfotransferase in physiological conditions are PAP and PAPS, respectively. Adenosine 5′-diphosphate (ADP)-, adenosine 5′-triphosphate (ATP)-, and 2′,5′-bisphosphate (2′,5′-PAP)-agarose have been used for the analysis of the binding of flavonol 3-sulfotransferase and its mutants.[31] However, previous studies have suggested that various nucleotides bind tightly to phenol sulfotransferase (PST) and some nucleotides, 2′,5′-PAP, adenosine 5′-monophosphate (AMP), and adenosine 2′:3′-cyclic phosphate 5′-phosphate (2′:3′-cyclic PAP) can be used by PST to replace PAP.[27] These experimental results provide a good basis on which to evaluate the docking method and force field parameters used and to predict the substrate specificity of phenol sulfotransferase.

Nucleotide inhibitors (such as ADP, ATP) of PST catalyzed physiological reactions (Reaction 1) have been reported in the literature.[32-34] The end product, PAP, and the structure analogues of PAPS, ADP, and ATP, have been shown to inhibit competitively the sulfation of PST when PAPS is the varied substrate.[33,34] However, our data suggests that these nucleotides are not inhibitors of the transfer reaction (Reaction 2) or the reverse physiological reaction catalyzed by PST. Specifically, we report our results on the docking of nucleotides to estrogen sulfotransferase (EST). These observations are in good agreement with the experimental data reported here. In this paper, we will focus on the application of one particular docking method and the parameters used to study substrate-enzyme interactions of sulfotransferase.

EXPERIMENTAL

In this paper, we used the X-ray crystal structures of EST (1aqu)[17] with the bound substrate PAP (equivalent to A3P as defined by PDB) and EST (1hy3)[22] with the bound substrate PAPS (equivalent to PPS as defined by PDB) as templates to develop the docking protocol that was used on all docking calculations.

The edited PDB crystal structure (with water molecules, PAP and 17β-estradiol) of EST (1aqu)[17] was imported and all possible hydrogen atoms were added. The same process was performed on the PDB crystal structure (with water molecules, PAPS) of EST (1aqu).[22] Considering that the active site of EST (1aqu) might be biased to PAP due to the possible conformational change, the substrates were docked into the X-ray structure of EST (1hy3), which was not a complex with a substrate.

Docking Tool: GEMDOCK

We used an automatic program, GEMDOCK (Generic Evolutionary Method for molecular DOCKing) modified from our previous work,[35] for docking flexible molecules. Our program used a simplified scoring function and a new evolutionary approach which is more robust than standard evolutionary approaches, including genetic algorithms and evolutionary programming. Our energy function consisted only of steric and hydrogen-bonding terms with a linear model which was simple and fast enough to recognize potential complexes. In order to balance exploration and exploitation, the core idea of our evolutionary approach is to design multiple operators cooperating with each other by using the family competition which is similar to a local search procedure.

GEMDOCK used a simple scoring function modified from Gehlhaar et al.[36] and our previous study.[35] This function, which is specifically designed for fast docking applications and comprises four terms: the non-bonded interaction and the electrostatic energy between ligand and receptor, a ligand internal energy associated with torsion angles, and a term penalizing solutions (E_{penal}) which lie outside a box defining the active site, is given

$$E_{tot} = \sum_{i=1}^{lig}\sum_{j=1}^{rec}\left[F_{ij}(B_{ij}(r_{ij})) + 332.0\frac{q_i q_j}{\tau(r_{ij})r_{ij}}\right] - E_{penal}$$

where B_{ij} are the nonbonded parameters for hydrogen binding and steric energy, $\tau(r_{ij})$ is the distance-dependent dielectric

constant, r_{ij} is the distance between the atoms i and j; q_i and q_j are the point charges of the atoms in the ligand and receptor, respectively; and 332.0 is a factor that converts the electrostatic energy into kilocalories per mole. The *lig* and *rec* denote the numbers of the atoms in the ligand and receptor, respectively. Because we did not include crystal water molecules or add solvent water molecules in our calculations, the distance-dependent dielectric constant ($\tau(r_{ij})=4r$) was employed to mimic the solvent effect during the calculations.

The non-bonded interaction term, $F(B_{ij}(r_{ij}))$, is a pairwise sum over ligand and protein heavy atoms, each term taking the piecewise linear form with six parameters, V_1–V_6 shown in Fig. 1, where r_{ij} is the distance between the atoms i and j; B_i is either hydrogen binding or steric state. In this atomic pair-wise model, the interactive types are only hydrogen binding and steric potential which have the same function form but with six different parameters. The parameters of a hydrogen bond interaction are 2.3, 2.6, 3.1, 3.6, -2.5, and 20.0 and the six parameters of a steric are 3.4, 3.6, 4.5, 6.0, -0.4, 20.0. Each heavy atom is assigned one of four different atom types (Table 1): donor, acceptor, both, or nonpolar. The hydrogen binding is formed by one of the following pair atom types: donor-acceptor (or acceptor-donor), donor-both (or both-donor), acceptor-both (or both-acceptor), or both-both. Other pair-atom combinations are to form the steric state.

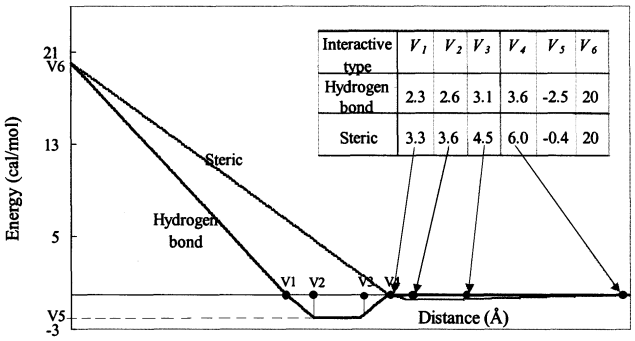


Fig. 1. The linear energy function form and six parameters of the pair-wise atom of the hydrogen bond and steric state in GEMDOCK.

We briefly describe the general steps of an evolutionary algorithm for solving flexible nucleotides docking problem in the following. First GEMDOCK fixes the location of the receptor site and randomly generates a starting population with N solutions by initializing the orientation and conformation of the nucleotide relating to the center of the receptor. Each solution is represented as a set of four n-dimensional vectors (x^i, σ^i, ν^i, ψ^i), where n is the number of adjustable variables of a docking system and $i = 1, \ldots, N$ where N is the population size. The vector x represents the adjustable variables to be optimized in which x_1, x_2, and x_3 are the 3-dimensional location of the nucleotides; x_4, x_5, and x_6 are the rotational angles; and from x_7 to x_n are the twisting angles of the rotatable bonds inside a ligand. σ, ν, and ψ are the step-size vectors of decreasing-based Gaussian mutation, self-adaptive Gaussian mutation, and self-adaptive Cauchy mutation. In other words, each solution x is associated with some parameters for step-size control. The initial values of x_1, x_2, and x_3 are randomly chosen from the feasible box, and the others, from x_4, x_5, and x_6, are randomly chosen from 0 to 2π in radians. The initial step size σ is 0.8 and ν, and ψ are 0.2. After GEMDOCK initializes the solutions, GEMDOCK enters the main evolutionary loop which consists of three main stages in every iteration: decreasing-based Gaussian mutation, self-adaptive Gaussian mutation, and self-adaptive Gaussian mutation. These mutation operators are described in our previous paper (35). Each stage is realized by generating a new quasi-population (with N solutions) as the parent of the next stage. These stages apply a general procedure "*FC_adaptive*" with only different working populations and the mutation operator.

The *FC_adaptive* procedure employs two parameters, namely, the working population P, (with N solutions) and mutation operator (M), to generate a new quasi-population. The main work of *FC_adaptive* is to produce offspring and then conduct the family competition. Each individual in the population sequentially becomes the "family father." With a probability p_c, this family father and another solution that is randomly chosen from the rest of the parent population are used as parents for a recombination operation. Then the new off-

Table 1. 'GEMDOCK Atom Type' and 'Heavy Atom Type'

GEMDOCK atom type	Heavy atom name
Donor	Primary and secondary amines, sulfur, and metal atoms
Acceptor	Oxygen and nitrogen with no bound hydrogen
Both	Structural water and hydroxy1 groups
Nonpolar	Other atoms (such as carbon and phosphorus)

spring or the family father is operated on by a mutation. For each family father, such a procedure is repeated L times called the family competition length. Among these L offspring and the family father, only the one with the lowest scoring function value survives. Since we create L children from one "family father" and perform a selection, this is a family competition strategy. This method avoids the population prematureness but also keeps the spirit of local searches. Finally, the *FC_adaptive* procedure generates N solutions because it forces each solution of the working population to have one final offspring.

GEMDOCK parameters and computational details

The GEMDOCK parameters were selected after many attempts to predict conformations for test proteins with various initial values. GEMDOCK optimization stops when either the convergence is below a certain threshold value or when the iterations exceed a preset maximum value. GEMDOCK searches on the 3-dimensional docking box with 12 Å, and the unique orientations differ by 0.2 Å and unique orientation angles differ by 0.03 radius from x_4 to x_6. The conformations of the single bonds (from from x_7 to x_n) differ by 0.03 radius. Therefore, the size of the search space is about 10^{28} if the number of single bonds of a ligand is 8.

RMSD Calculations and Values

Root-mean-square derivation (RMSD) of heavy atomic positions was used to assess the accuracy of the prediction. The RMSD was calculated using the formula

$$\left\{\sum_{i=1}^{M}\left[(X_i - x_i)^2 + (Y_i - y_i)^2 + (Z_i - z_i)^2\right]/M\right\}^{1/2}$$

where M is the number of atoms in a nucleotide and (X_i, Y_i, Z_i) and (x_i, y_i, z_i) the coordinates of the ith atom of the reference X-ray crystal and the predicted structure, respectively. The calculation of RMSD included only the heavy atoms of a nucleotide.

RESULTS AND DISCUSSION

GEMDOCK as a tool for the molecular docking of nucleotides

In order to evaluate GEMDOCK, we started with two known PST structures, 1aqu and 1hy3 from PDB that bind to PAP and PAPS, respectively. Using GEMDOCK, PAP and PAPS were docked successfully into active sites of 1aqu and 1hy3 with RMSD of 0.51 and 0.41 Å, respectively. Figs. 2c and 4e and Table 2 show the docked results and confirm that GEMDOCK is able to obtain the good predicted structures.

Next we docked all of the tested nucleotides, including PAP, PAPS, AMP, ADP, and ATP, into the active sites of both 1aqu and 1hy3. Figs. 2 and 4 and Table 2 show the best docked results. The reference molecules are PAP and PAPS for 1aqu and 1hy3, respectively, when we calculated the RMSD values for all tested nucleotides. According to the RMSD values obtained, these docked structures for both sulfotransferases can be roughly divided into two categories. Table 2 shows the RMSD values of PAP, PAPS, AMP, ADP, and ATP that are less than 2.0 Å for the sulfotransferase 1aqu and 1hy3. The other category of RMSD values that are much more than 2.0 Å (docked results shown in Figs. 3 and 5) are

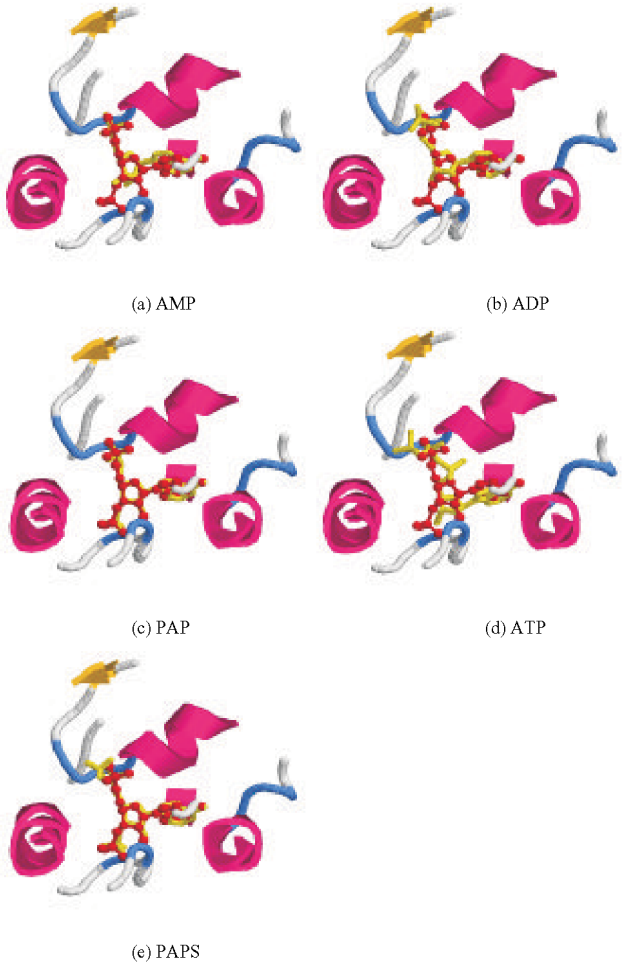


Fig. 2. The best docked first category conformations of nucleotides with 1aqu. The X-ray structure of PAP in 1aqu is shown as ball-and-stick. The best docked structures of nucleotides are shown as yellow lines. Five nucleotides used for the docking are (a) AMP, (b) ADP, (c) PAP, (d) ATP, and (e) PAPS.

Table 2. The Results of Nucleotide Docking on 1aqu and 1hy3 with GEMDOCK

	PAP	PAPS	AMP	ADP	ATP
1aqu					
Minimization Energy of docked structure (cal/mole)	-192.12	-219.96	-151.03	-154.32	-193.83
RMSD[a] (Å)	0.51	1.06	1.25	0.76	1.85
1hy3					
Minimization Energy of docked structure (cal/mole)	-192.32	-222.97	-149.72	-173.03	-194.60
RMSD[b] (Å)	1.20	0.41	1.28	1.44	1.96

[a] The values were calculated using reference molecule PAP for these tested nucleotides.
[b] The values were calculated using reference molecule PAPS for these tested nucleotides.

not given. Figs. 3 and 5 simply show that alternative binding conformations were also predicted. This was an important finding that may explain the puzzling question as we discuss further the binding and inhibition of sulfotransferase by nucleotides.

Variation of sulfotransferase complexes as template for the molecular docking of nucleotides

Nucleotide (PAP) binding induces conformational change of sulfotransferase.[16] X-ray structures of sulfotransferases also confirm that amino acid ligands of nucleotides varied with PAP or PAPS.[17-22] To distinguish this difference, two sulfotransferase structures, 1aqu with the bound substrate PAP and 1hy3 with the bound substrate PAPS, were used as templates to develop the docking protocol that was used on all docking calculations. The best docked structures are shown in Figs. 2 and 3 or Figs. 4 and 5 when 1aqu or 1hy3, respectively, were used as templates.

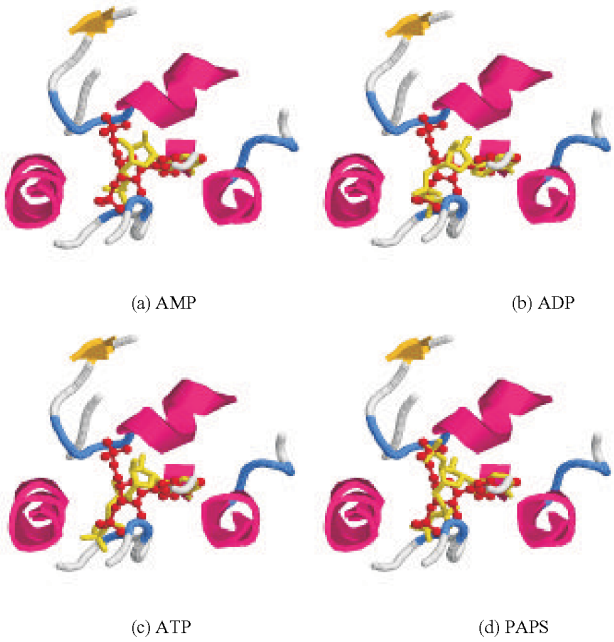


Fig. 3. The best docked second category conformations of nucleotides with 1aqu. The X-ray structure of PAP in 1aqu is shown as ball-and-stick. The best docked structures of nucleotides are shown as yellow lines. Four nucleotides used for the docking are (a) AMP, (b) ADP, (c) ATP, and (d) PAPS.

Two categories of the best docking results were obtained (Figs. 2 and 4 belong to the 1st category and Figs. 3 and 5 belong to the 2nd category). These two categories gave similar minimization energy of docked structure but were distinguished by their significant difference in RMSD values as compared to those of their X-ray crystal structures. The best docked structures of nucleotides are shown as yellow lines in contrast to the red molecule that was co-crystallized with the enzyme. A very well matched orientation of the red and yellow molecules were obtained as shown in Figs. 2 and 4 and were classified as the 1st category. Their minimization energies and RMSDs are shown in Table 2. These data confirmed that the predicted binding of the tested nucleotides were docked in a similar environment. This result was expected for they are similar in structure. However, as shown in Table 3, *Ki* of these tested nucleotides were found significantly different for the physiological reaction catalyzed by sulfotransferase. This is one of the questions that we would like to explain and will be discussed further in the next section. The

Table 3. Inhibition Constants for the Competitive Inhibition of Sulfotransferase by Nucleotides[a]

pNP + PAPS → pNPS + PAP
↓↑
Inhibitors

Inhibitors	K*i* (μM)
PAP	0.1 ± 0.01
AMP	> 1000
ADP	30.0 ± 1.2
ATP	23.2 ± 0.7

[a] Data obtained from Rens-Domiano and Roth. (Ref. 34)

structures and binding constants of the tested nucleotides are shown in Table 4. All the binding constants are in the same ranges except for PAPS. It is worth noting that the binding constants were obtained from a sulfotransferase that was not complex with any other nucleotide, i.e., the conformation may not be the same as that of the X-ray crystal structures that we used as templates for the nucleotide molecular docking. The data shown in Table 4 confirm that nucleotides are tightly bound to sulfotrasnferase except for PAPS.

On the other hand, for the sulfotransferase 1aqu, the AMP, ADP, ATP and PAPS clustered into another category are shown in Fig. 3. Similarly, for the sulfotransferase 1hy3, the AMP, ADP, PAP and ATP clustered into another category are shown in Fig. 5. These results indicated that there could be an alternate binding conformation for the tested nucleotides with sulfotransferase. It was interesting to observe that the difference of orientation of the predicted docking was about 180 degrees for the 1st and 2nd categories of nucleotide conformation. The use of different templates, 1aqu or 1hy3, did not seem to significantly affect the predicting results.

Implication of nucleotide conformation in sulfotransferase as predicted by GEMDOCK

Several nucleotides (such as PAP, AMP, ADP and ATP) were shown to inhibit competitively the sulfation catalyzed by PST when PAPS is the varied substrate (Table 3). Previous

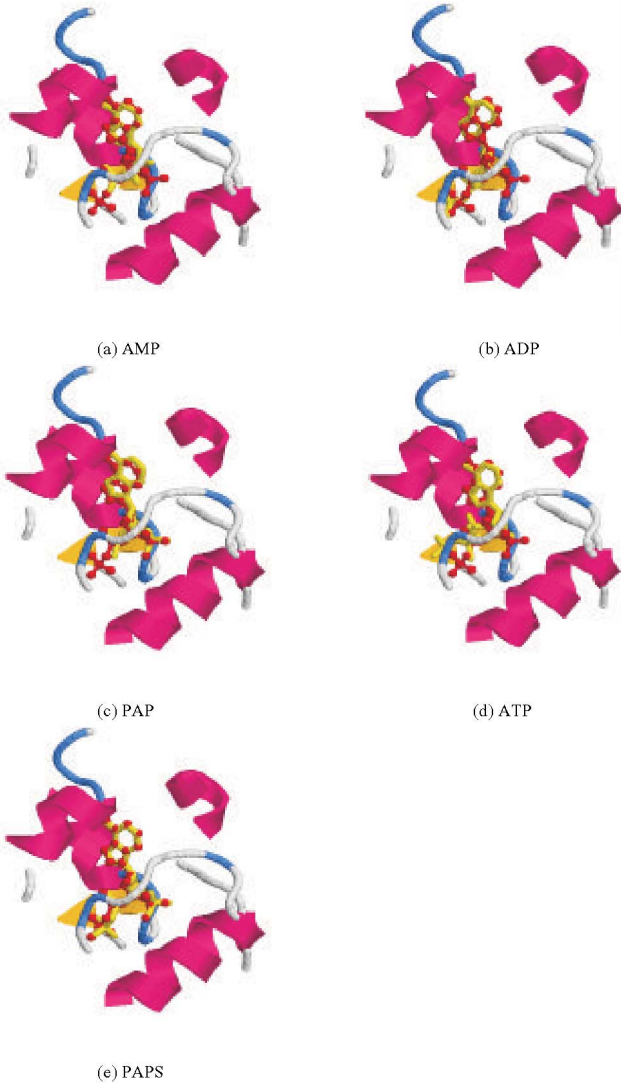


Fig. 4. The best docked conformations of nucleotides with 1hy3. The X-ray structure of PAPS in 1hy3 is shown as ball-and-stick. The best docked structures of nucleotides are shown as yellow lines. Five nucleotides used for the docking are (a) AMP, (b) ADP, (c) PAP, (d) ATP, and (e) PAPS.

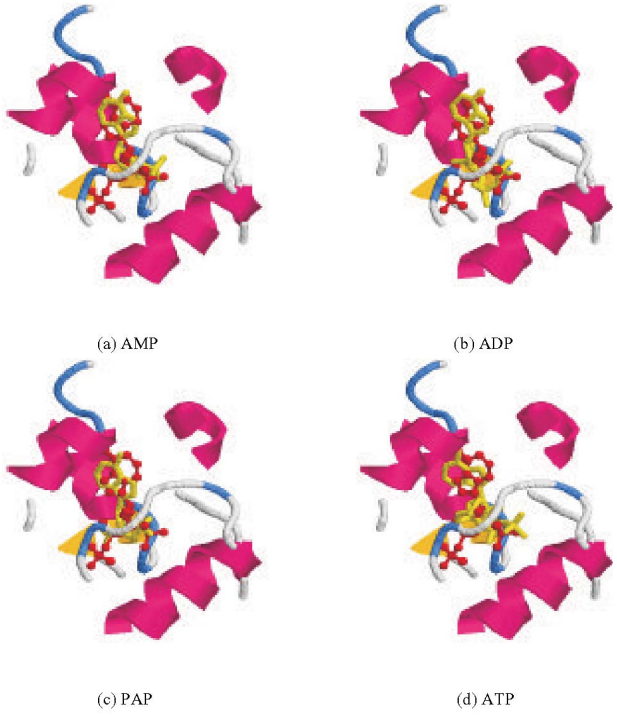
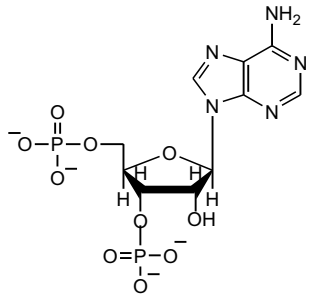
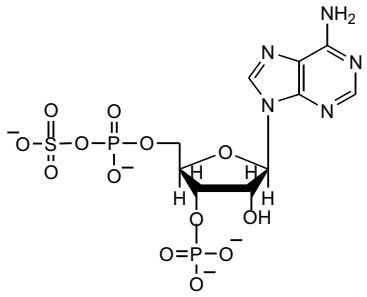
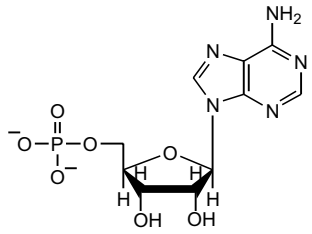
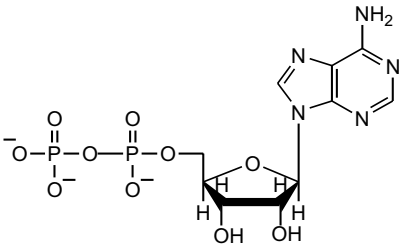
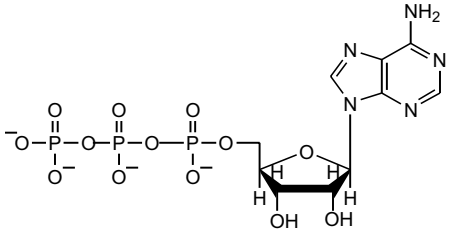


Fig. 5. The best docked second category conformations of nucleotides with 1hy3. The X-ray structure of PAPS in 1hy3 is shown as ball-and-stick. The best docked structures of nucleotides are shown as yellow lines. Four nucleotides used for the docking are (a) AMP, (b) ADP, (c) PAP, and (d) ATP.

Table 4. Nucleotides Used for the Docking in PST

Name	Structure	Dissociation constants		Abbreviation
		Kd1 (nM)	Kd2 (μM)	
3′-phosphoadenosine 5′-phosphate		31 ± 4[a] 20[b]	152 ± 1[a] 200[b]	PAP
3′-phosphoadenosine 5′-phosphosulfate		>>[b]	>>[b]	PAPS
Adenosine 5′-monophosphate		9.2 ± 0.3[a]	164 ± 4[a]	AMP
Adenosine 5'-diphosphate		38 ± 1[a]	75.0 ± 0.3[a]	ADP
Adenosine 5′-triphosphate		79 ± 1[a]	122 ± 1[a]	ATP

[a] Data obtained from Lin and Yang. (Ref. 26)
[b] Data was too large to be measured. (Ref. 24)

studies also showed that various nucleotides bind tightly to PST (Table 4), and some nucleotides, 2′,5′-PAP, AMP, 2′:3′-cyclic PAP can be used by PST to replace PAP.[27] Consistent with the previous findings, the predicted docking of

various nucleotides also gave satisfactory results (especially the 1st category binding). All of this information seems to straightforwardly explain the inhibition and binding of nucleotides. Two important pieces of information indicate that the binding of nucleotides with sulfotransferase may be more complex that we have expected. Our experimental data suggests that these nucleotides are not inhibitors of the transfer reaction or the reverse physiological reaction catalyzed by PST (data not shown). Table 3 also shows that the nucleotide as an inhibitor can be very much different in their efficiency as indicated by their significant variation in *Ki*s. We have proposed that different binding sites may be used for different purposes for there are at least two available binding sites for nucleotides in a sulfotransferase dimer.

To find an explanation for the above experimental data, we may use our results on the docking of nucleotides to EST. These observations were in good agreement with the previous experimental data. The docking results (summarized in Table 2) explain why all the nucleotides tested bind tightly to PST (*Kd*s at nM range as shown in Table 4). It also explains why some nucleotides may replace PAP as substrates for PST (for the docking results of the 1st category). The 2nd category of nucleotide binding reveals a possible alternate nucleotide binding mode for the inhibition of sulfotransferase activity. This may explain the differences in inhibition for the physiological reactions, the transfer reactions and the reverse physiological reactions. Since different binding sites and different binding conformations may exist, binding of nucleotides does not necessarily result in the inhibition of the sulfotransferase catalyzed reaction. It is also possible that the transfer reaction catalyzed by sulfotransferase uses different binding sites from those of the physiological reactions. This may explain why only a catalytic amount of PAP is required for the transfer reaction.[25] By comparing these results with binding sites and binding compounds of the nucleotides with known EST X-ray structures (1aqu and 1hy3), it is possible to highlight the site specificity and the inhibition mechanism of the select compounds. The results presented here will be very helpful for the future study of these unknown mechanisms of sulfotransferase action.

Possible roles of nucleotides on the posttranslational modification and regulation of sulfotransferase

This research provided further binding conformation of nucleotides with a variety of nucleotides that may be abundant in the physiological condition. The binding site of a protein is usually very selective for a particular molecule and with a specific function. With the known biological function of sulfotransferase, the complexity of the binding of sulfotransferase with a variety of important nucleotides may indicate that regulation of biological function is involved. We have demonstrated that nucleotide binding plays an important role on the posttranslational modification of phenol sulotransferase.[37] The regulation of sulfotransferase is an important issue that has not been thoroughly studied. We expect that the use of molecular docking will be very useful for the further understanding of the molecular mechanism of the regulation of sulfotransferase.

CONCLUSION

GEMDOCK was found reliable for the prediction of PAP and PAPS binding with sulfotransferase as compared to their known X-ray crystal structures. Using GEMDOCK, PAP and PAPS were docked successfully into active sites of 1aqu and 1hy3 with RMSD of 0.51 and 0.41 Å, respectively. The tight binding of nucleotides was predicted by GEMDOCK with excellent agreement with previous experimental data. In this study, it was also confirmed from previous experimental data that PAP binds better than other nucleotides to sulfotransferase. Alternative nucleotide binding conformations were also predicted by GEMDOCK. This result gave a clue for the further study of the mechanism of the inhibition of the sulfotransferase that cannot be explained with previous models.

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