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# Research Report

## On the Nucleoside Binding of Herpes Simplex Type 1 Thymidine Kinase: A Theoretical Study

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# On the Nucleoside Binding of Herpes Simplex Type 1 Thymidine Kinase: A Theoretical Study

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**Abstract**

We present a theoretical study of the interactions of viral herpes simplex type 1 thymidine kinase with its substrate, thymidine. Density functional theory calculations with gradient-corrected exchange-correlation functionals are carried out for various models of the nucleoside binding site–substrate adducts. Our calculations indicate that (i) Coulombic interactions play an important role in the substrate binding, (ii) the role of Met 128, an important residue in the active site cavity of the enzyme, is purely steric and hydrophobic, in agreement with very recent site-mutagenesis experiments (Folkers, G. *et al.*, in preparation), and (iii) transferring a proton from Tyr 172 in the active site to the substrate may be a key step in the enzyme's mechanism of action.

## I. INTRODUCTION

Various applications in the treatment of virus infections and in cancer therapy are based on the viral herpes simplex type 1 thymidine kinase (HSV1 TK).[1–13] HSV1 TK catalyzes the transfer of the $\gamma$-phosphate group from ATP to the 5'-OH group of thymidine (ATP-thymidine-5'-phosphotransferase, EC 2.7.1.21). Its physiological role is to salvage thymidine into DNA metabolism by converting it into thymidine monophosphate.

In contrast to various other thymidine kinases (for example the human isoenzyme), HSV1 TK shows extremely large substrate diversity.[14,15] The nucleoside and nucleoside-analogue substrates consist of pyrimidine (thymidine,[16,17] iodeoxyuridine[18]) and purine (acyclovier,[19–21] ganciclovir[19,20,22]) base rings, and the ribose moiety can be substituted by acyclic side chains.

The X-ray structure of HSV1 TK complexed with ATP and thymidine has been determined at 3.0 Å resolution[23] and recently refined at 2.7 Å resolution. HSV1 TK is a dimeric enzyme with 376 residues per subunit. The two subunits are related by C2 symmetry. The active site is formed by an ATP and a nucleoside binding region.

A visual representation of the native substrate thymidine and the residues that play a role in the nucleobase fixation of HSV1 TK is given in Figure 1a. Thymidine is located between Met 128 and Tyr 172 in a sandwich-like orientation. A complex H-bond network is present in the active site: the thymine ring interacts with the amide group of Gln 125 via O2 and H15 (see Chart I) and with two ordered water molecules via O4. The water molecules interact also with the guanidinium group of Arg 176. His 58 and Arg 163 both interact with the hydroxyl group of Tyr 172.

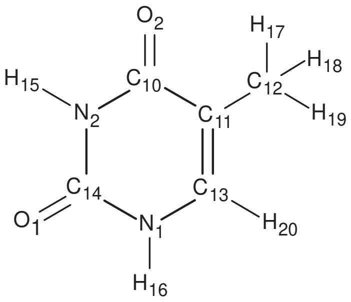


**Chart I**

We present here a density functional theory (DFT) investigation[24] of the interaction of a model of the HSV1 TK active site with the nucleoside. Our aim is to gain a better understanding of the nature of HSV1 TK binding interactions and of its mechanism of action. This may facilitate the development of more specific prodrugs and is a crucial requirement for successful protein engineering of HSV1 TK.

Our calculations indicate that (i) binding is dominated by Coulombic interaction, (ii) the role of Met 128 in substrate fixation is purely hydrophobic, and (iii) the binding energy is lowered by transferring a proton from Tyr 172 to the substrate.

## II. COMPUTATIONAL SECTION

**Modeling.** We will focus here on the nucleoside binding region, which is peculiar to this enzyme.

The binding of HSV1 TK to the sugar moiety of the nucleoside is not very specific.[25] The nucleotide–enzyme interaction plays a more crucial role in the binding and recognition of HSV1 TK. Thus it is the focus of the present paper.

Our modeling of HSV1 TK active site–nucleoside adduct (Figure 1a) includes Met 128 [modeled by dimethylsulfide (dms)], Tyr 172 (modeled by cresol), Arg 163 (modeled by an ammonium ion) and the substrate thymidine (modeled by thymine) (see Figure 1b).

Several complexes have been considered by protonating the residues and the substrate differently. We will now describe our model in detail.

*Thymine.* Various tautomeric forms for the neutral and protonated nucleobase have been considered (see Chart II).

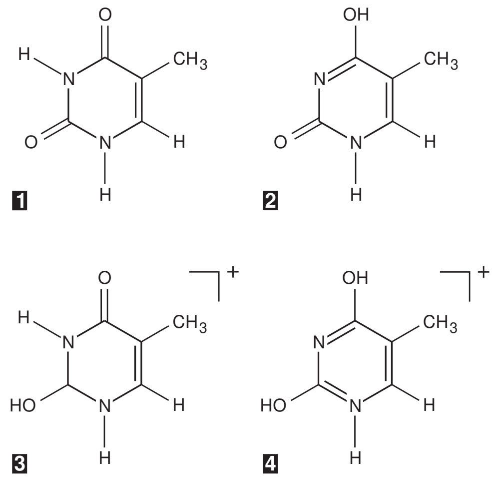


**Chart II**

*Met 128 and Tyr 172.* These residues obviously play a fundamental role in the fixation of the substrate, and their entire side chains are included. For Tyr 172, we considered not only the neutral but also the deprotonated form. Indeed, Tyr 172 H-bonds to His 58 (not included in our model) and may transfer a proton to the N$\epsilon$ atom.[26] The resulting deprotonated form would be stabilized by Arg 163 (N$\eta X - \mathrm{O} = 2.9$ Å).

*Arg 163.* This residue is important for catalytic activity[27] because of its electrostatic role and because its guanidinum group stabilizes the position of Tyr 172. This interaction was taken into account by modeling the guanidinum of Arg 163 as an ammonium ion, analogously to other quantum chemistry calculations.[24b,28]

*Gln 125.* This residue was not included because (i) its H-bond interactions with the substrate (via its amide group) are very similar for all the complexes studied here and presumably will add a similar stabilization energy, and (ii) the activity ($k_{cat}$) of the Q125L HSV1 TK mutant is similar to that of the wild-type HSV1 TK.[29]

*His 58.* Although it was not added in our complexes, we have included one possible effect of this residue by deprotonating Tyr 172.

*Water molecules.* We have considered one of their possible effects by protonating the thymine in position O1 (see Chart I).

Here is a summary of the complexes under consideration in this paper:[30]

| | | |
|---|---|---|
| **I**: | thymine(1), cresol, dms, ammonium | (total charge: +1) |
| **II**: | thymine(3), cresolate, dms, ammonium | (total charge +1) |
| **III**: | thymine(4), cresolate, dms, ammonium | (total charge: +1) |
| **IV**: | thymine(2), cresol, dms, ammonium | (total charge: +1) |
| **V**: | thymine(3), cresol, dms, ammonium | (total charge: +2) |
| **VI**: | thymine(1), cresolate, dms, ammonium | (total charge: 0) |

**Calculations.** Our calculations were performed within the framework of density functional theory (DFT) and use the generalized gradient approximation. All the calculations were performed with a scalar version of the Car–Parrinello[31] code developed by J. Hutter, 1993-1995.

The parametrizations of the functionals for exchange and correlation are those of Becke[32] and Lee–Yang–Parr[33], respectively. The interaction between valence electrons and ionic cores is described by pseudopotentials of the Martins–Troullier type[34]. The Kohn–Sham orbitals are expanded in plane waves up to an energy cutoff of 70 Ry.

We have carried out geometry optimization using the direct inversion in iterative subspace method (DIIS)[35,36] for both electronic and ionic degrees of freedom. We have used nonperiodic boundary conditions as in Ref. 37. A position constraint was imposed for the C$\beta$ of Tyr 172 and Met 128 and on the ammonium nitrogen.

The complexes were constructed starting from the X-ray structure of HSV1 TK[23] and optimized in a cubic box of 15.8 Å. The root mean square displacements of the optimized structures were all in the range 0.12–0.15 Å.[38]

In order to check the stability of the complexes investigated here we have also carried out DFT-based molecular dynamics simulations.[31,39] The simulations were performed for complexes **I** and **III**, with and without using the position constraints mentioned above. The trajectories were collected over a period of only 0.5 ps. The equations of motion were integrated using the Verlet algorithm with a fictitious electronic mass of 500 a.u. and an integration time step of 0.121 fs. We have used the Nosé thermostat on the ions.[40] The target temperature was set to 298 K and the frequency of the thermostat to 500 cm$^{-1}$.

## III. RESULTS

In this section we will present the results of our calculations, focusing on the energetics and on the nature of the enzyme–substrate binding.

**Energetics.** As expected, the total energies of the complexes decrease with increasing charge, e.g. with increasing number of protons.[41] However, we have to consider that a proton transfer from the solvent (water and/or protein frame) to the HSV1 TK active site will increase the energy of the solvent. Therefore, comparison of energies of complexes bearing different charges is difficult, and it is more meaningful to compare binding energies, defined as the differences between total energies and the energies of the substrate and the binding pocket (see Figure 1, Table I).[42] Table I shows that complex **V**, in which thymine is protonated, is by far the most unstable system investigated here.[43]

A general result of our calculations is that the deprotonation of Tyr 172 results in energy stabilization, mainly due to favorable Tyr 172–thymine interactions.[44] Indeed, the complexes in which only dipole/dipole interactions with the substrate are present (complexes **I** and **IV**) are less stable than **VI**, in which charge/dipole interactions exist. The most stable complexes are **II** and **III**, in which the deprotonated Tyr interacts with the protonated thymine. These are also the only complexes whose binding energy is negative.

The stability of the complex **I** and III was further investigated including temperature effects by carrying out DFT-based molecular dynamics simulations (see Computational section). As for the optimization procedure, we have constrained the position of the $\beta$-carbon atoms of Tyr 172 and Met 128 and the ammonium nitrogen. As an example, Figure 2 shows the initial and final structure of complex **I** superimposed. Note that no major rearrangement of the substrate occurs on the simulated time scale (0.5 ps). This was observed also for complex **III**. Instead, carrying out the dynamics without position constraints resulted in a complete disruption of both sandwich complexes.

A comment is in order concerning the fact that DFT does not reproduce the dispersion attraction[45] of the Tyr 172–thymine and thymine–Met 128 interactions. However, owing to its high unspecificity, the dispersion energies are expected to be nearly the same and to cancel out with respect to their binding energy differences. In addition, they are much smaller in magnitude than electrostatic energies, which appear to be very important in our complexes.

**HSV1 TK active site–substrate binding.** Using the DFT approach, we have investigated the nature of substrate/enzyme binding by means of the difference density $\Delta\rho = \rho_{\text{complex}} - \rho_{\text{fragments}} - \rho_{\text{substrate}}$. The difference density describes how the electron density $\rho$ changes during the formation of the complex. Figure 3 shows that no charge transfer from or to the substrate is present in complex **I**. We observe instead a certain polarization of the electronic states. This is characteristic for all the complexes studied here. We find strong polarization of the O and N atoms of thymine, and of the H-bond of the ammonium–cresole/cresolate interaction. Interestingly, there is no polarization effect of the Met 128 sulfur. This indicates that sulfur plays only a minor role in binding.

We have further investigated the role of Met 128 by plotting the Kohn–Sham levels and the density of states (DOS) at the HOMO–LUMO gap region. Figure 4 shows the results for complex **III**. We note that the states of Met 128 hardly overlap with those of the substrate, as clearly shown by the DOS projected on the two individual molecular fragments. Analogous results have been found for all the other complexes studied here. The Met 128 sulfur–substrate interaction appears to be purely hydrophobic and steric in nature.

## IV. DISCUSSION AND CONCLUSION

Our calculations indicate that in the formation of the HSV1 TK–thymine adduct, the binding is dominated by Coulombic interactions. In addition, they show that charge transfer does not take place to or from the substrate.

The role of Met 128 sulfur in the binding process appears to be purely hydrophobic and steric. Its nonspecific role has been confirmed by very recent site-directed mutagenesis experiments, which have shown that the activity is preserved when the Met residue is replaced by another hydrophobic residue such as an Ile.[46] Calculations and experiments indicate that protein engineering experiments on this position might increase the affinity of the enzyme to nucleosides.

Our calculations predict that the stable complexes are those bearing an internal "proton transfer" complex, i.e. negative, deprotonated Tyr 172 and positive, protonated substrates (complexes **II** and **III**). This is consistent with site-directed mutagenesis experiments, which have indicated an extreme dependence of the affinity of the substrate on changes in the electrostatics at the active site. Indeed, replacing Gln 125 (Figure 1a) with a Glu residue (thus introducing a negative charge at the active site) results in a loss of biological activity.[29] Instead, substitution with a neutral leucine in the same position results in similar activity as that of a wild type HSV1 TK.[29]

The postulated proton transfer complex could play an important role in the enzyme's mechanism of action. The rigidity of the active site, and thus the catalytic activity of the enzyme, would be increased by the strong ionic interaction. In addition, the highly favorable thymine–enzyme interaction may render the enzyme–sugar interaction less crucial for the binding, consistent with experiment. Indeed, HSV1 TK is able to phosphorylate nucleoside analogues with acyclic side chains such as the widely used drugs acyclovir,[19,20] ganzyclovir,[19,20] and the alternative substrate 2-HM-HBG.[25] Finally, such an unspecific ionic interaction is consistent with the diversity of the nucleobase binding of HSV1 TK and of such other kinases as herpes simplex virus type 2 and bovine herpes virus type 1 TKs, in which the combination of His and Tyr in the active site is also conserved;[47] in all the other kinases,[48] characterized by relatively strict substrate acceptance,[47] the highly hydrophobic Phe residue replaces Tyr 172 in the active site.

Our calculations suggest a specific role of the electrostatic of the active site cavity in the mechanism of action of HSV1 TK. Indeed, the protein frame electrostatics could stabilize the huge dipole of the Tyr 172–thymine ion pair. This stabilization could be highly reduced once the substrate is phosphorylized, because the conformation of the active site is expected to change significantly. Therefore, the electrostatic produced by HSV1 TK could be important both in substrate fixation and in product release.

We plan to test this hypothesis by including the effect of the electrostatic environment in the quantum-chemical calculations.

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[38] As test calculations, the structures of some of the individual fragments, namely $NH_4^+$, cresole and thymine, were optimized. Good agreement with experiment was obtained (data available upon request).

[39] For a review of the method see, e.g., Galli, G., Parrinello, M. in *Computer Simulation in Material Science*, NATO ASI Series E, Vol. 205; Meyer, M. Pontikis, V. Eds.; Kluwer: Dordrecht, 1991.

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[41] Adding one proton to the system resulted in an energy gain of about 200 kcal/mol.

[42] Solvation and hydrophobic effects should be included in the discussion of realistic binding energies. However, in all complexes investigated here the substrate is thymine and therefore these effects are expected to be nearly constant.

[43] This is also shown by the higher protonation energy (about 30 kcal/mol) of thymine in the presence

of HSV1 TK with respect to the isolated case.

[44] Indeed, the deprotonation energy of cresole turns to be about 100 kcal higher *in vacuo* than in the field of the HSV1 TK active site.

[45] See, e.g. Kristyán, S.; Pulay, P. *Chem. Phys. Lett.* **1994**, *229*, 175; Pérez-Jordán, J.; Becke, A. *Chem. Phys. Lett.* **1995**, *233*, 134.

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TABLE I. Binding energies of complexes **I–VI** (kcal/mol), calculated as $E_{\text{binding}} = E_{\text{total}} - E_{\text{substrate}} - E_{\text{activesite}}$. The differences of binding energy with respect to **III** are also reported.

| | | |
|---|---|---|
| **I** | +7.5 | (+27.9) |
| **II** | −10.0 | (+10.4) |
| **III** | −20.4 | (0.0) |
| **IV** | +7.9 | (+28.3) |
| **V** | +37.0 | (+57.4) |
| **VI** | +3.4 | (+23.8) |

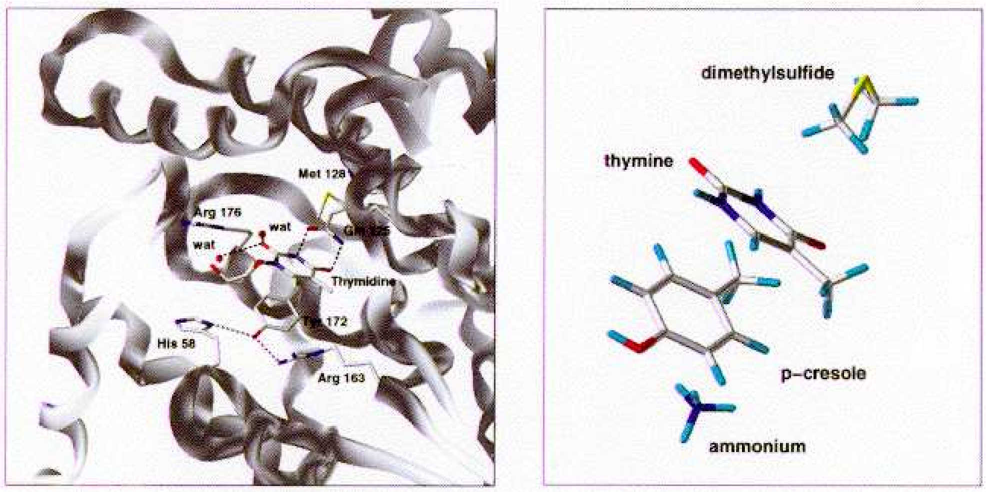


FIG. 1. (a) HSV1 TK active site and (b) model used in the calculations.

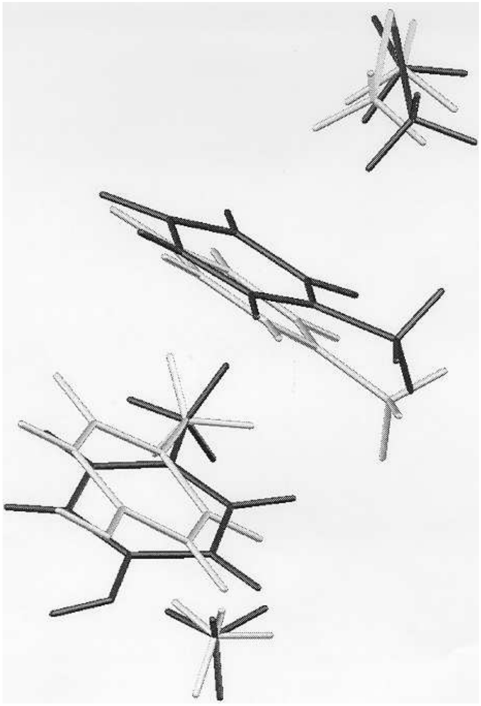

FIG. 2. Comparison of the starting structure of I (white) and the snapshot after 0.5 ps (black) of model **I**.

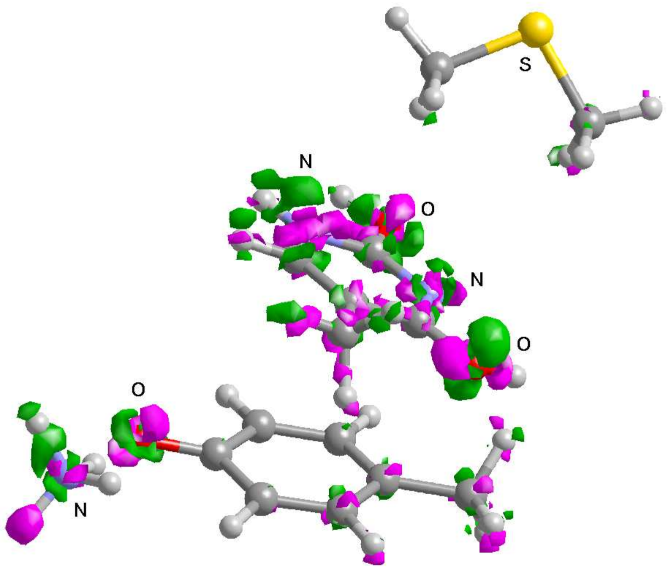


FIG. 3. Isodensity contour of the difference electron density for complex **III** $\Delta\rho$. Green: $+0.054e/\text{Å}^3$; magenta: $-0.054e/\text{Å}^3$.

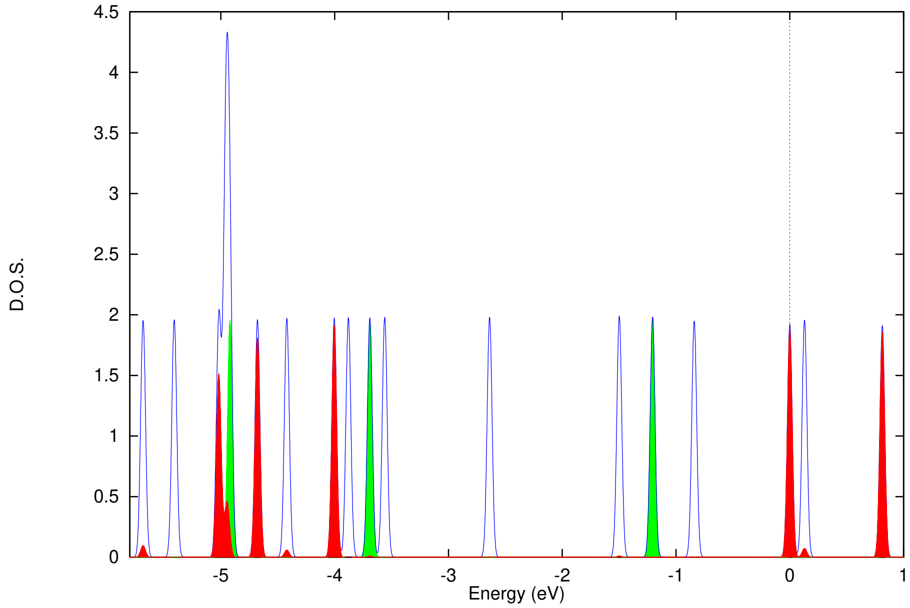


FIG. 4. Density of the highest occupied states and lowest unoccupied states of **III** smoothed with a Gaussian of width 0.05 eV: the green and red regions represent the contribution of Met 128 and thymine, respectively. $E = 0$ eV is the HOMO level.