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Molecular interactions in enzymes: a DFT approach Sugar binding to HSV TK

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1. Introduction

The thymidine kinase from herpes simplex virus type 1 (HSV-1 TK) (1) salvages thymine into the virus's metabolism by converting it to thymidine. HSV-1 TK acts as phosphorylating agent towards a large variety of nucleoside analogs, from acyclovir (ACV), to ganciclovir (GCV), penciclovir (PCV), anydro-idouracil (AHIU) and nor-methanacarbon ((N)-MCT) even though with much less efficiency than the natural substrate (Thymine). The striking substrate diversity of HSV-1 TK relative to the human isoenzyme (H TK) has been successfully exploited in the last two decades for antiviral and suicide gene-therapy based anticancer intervention (2). In particular, success of anticancer gene therapy is based on the phosphorilation of the nucleoside analogs, so the achievement of high catalytic efficacy becomes crucial.

While affinity has been quantitatively understood in terms of hydrogen bonds binding the drug to the enzyme active site, no clear explanation has been found for the measured catalytic constants (k_{cat}). Moreover, no rational explanation has been given for the role played by the sugar moiety in the enzymatic reaction.

A deep understanding of the nature of drug/target interactions could from one side help design of novel and more powerful (pro)drugs/protein and from the other provide a rationale for mutations causing drug-resistance and unfavorable side-effects (3).

Thanks to the large number of X-rays structures which recently have become available (4-6), it is now possible to dissect the key facets related to the phosphorylation process at the structural level.

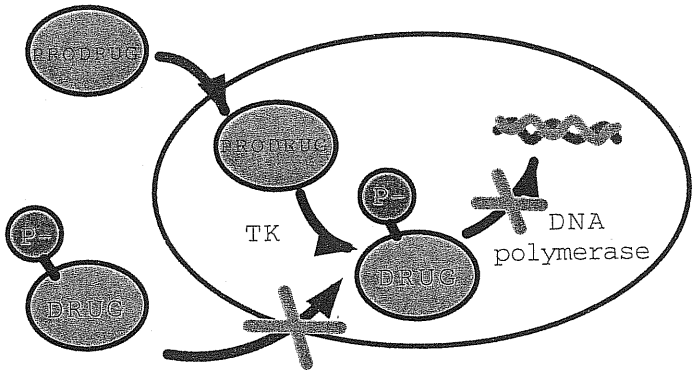
In this thesis I will concentrate in particular on the role played by sugar moiety which has not already been understood and I will try to relate the interaction energies to the catalytic properties. I will present a comparative study of TK active site in complex with the natural substrate and some of the drugs up to now used in antiviral and anticancer therapy. A combined experimental and theoretical approach, in collaboration with prof. G. Folkers' laboratory in Zurich permitted us to relate specific substrate-enzyme molecular interactions to the experimental values for k_{cat}. In particular we elucidated the role played by electrostatics giving an explanation for mutagenesis experiments on specific residues.

Our calculations were performed within a density functional theory approach to take into account electronic degrees of freedom which has been shown to play a relevant role for other biological systems. Models for the active site of drug-enzyme complexes are based on the crystallographic structures which have been recently resolved. Interaction energies between substrates and specific residues of the active site are compared to catalytic constants obtained for the same substrates in Folkers group laboratory in Zurich.

2. Gene therapy for Anticancer Research

2.1 Salvage pathways and role of TK.

Goal of anticancer gene therapy is to destroy the cancerous cells blocking their DNA replication. The simplest way would be to act directly on the DNA polymerase with three-phosphate nucleotides analogs. The main problem is that these charged drugs cannot overcome the cell membrane barrier so prodrugs are introduced in the cells. There they are transformed into the drugs by thymidine kinase (TK) provided by Herpes Simplex Virus (HSV) gene. Why HSVTK? Answer has been given by Elion and her cooworkes in 1977 (2) and is mainly based on two important properties of the viral TK: low substrate specificity and fast binding. The first characteristic permits to the viral kinase to bind and phosphorilate substrate analogs. These activated drugs are then processed by DNA polymerase and interpreted as chain terminals interrupting the DNA replication. A faster binding with respect to human TK reduces side effects due to the permanence of the drug in the human body.



Up to now the most widely used drug is ganciclovir but it has still a reduced activity and strong side effects. Goal of gene therapy research is to realize more specific drug-TK complexes to reach higher affinity (measured by K_m) and to increase phosphorilation rate (measured by k_{cat}).

On the experimental point of view research is working on the expression of new mutant and on the developing of new drugs with higher affinity, lower toxicity and more bioavaibility. On the teoretical point of view understanding of substrate-TK interactions, with the help of modern instruments as ab initio molecular dynamics, can give new hints for rational drug design.

2.2 Substrate diversity

In the following a will give a short description of the structure of the drugs which, up to now, have been crystalized in complex with the target enzyme and have been analyzed in the present work (see Fig.1). Natural substrate for TK is **deoxythymidine** (**dT**). It is composed by a nucleobase (thymine) and by a sugar mojety (deoxyribose). Thanks to its low selectivity HSV TK can phosporilate a wide range of nucleside analogues which can subsequently inhibit viral replication. Most well known antiviral is **acyclovir** (**ACV**) which is a purine nucleoside analogue with C2' and C3' missing. It is specifically phosphorilated at the position equivalent to the 5' hydroxile group. **Ganciclovir** (**GCV**) has the same acyclic chain as ACV, but with one more hydroxile group. **Penciclovir** (**PCV**) is identical to GCV except for a CH_2 substituting the oxygen at position 4'. These last three compounds exhibt the same nucleobase (guanine) but three different sugar mojeties. **Anhydro-dideoxy-ioduracil** (**AHIU**) has six membered ring in the place of

ribose, while the new compund, **nor-methana-carbon** (**(N)-MCT**), has a cyclopropane bridge on the ribose. All mentioned drugs act as fraudolent substrates for the viral kinase. **Hydroxy-butil-phenilguanina** (**HBPG**) is the only drug acting as inhibitor even if it shows only small differences with respect to acyclovir, the main one being a CH_2 replacing O4'.

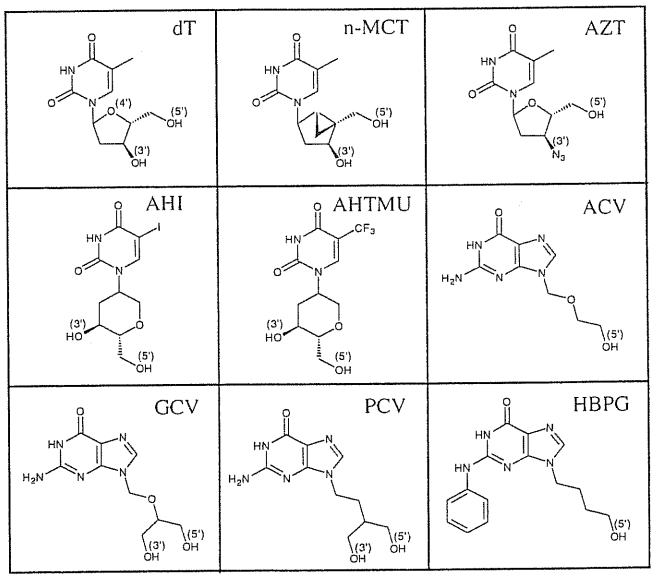


Figure 1. *Chemical formulas of selected (fraudulent) substrates and inhibitors of* TK_{HSV1}. *dT is the natural substrate; n-MCT, ACV, PCV, GCV, AHIU, AHTMU and AZT are prodrugs; and HBPG is an inhibitors. The 5'OH and 3'OH groups belonging to dT and their mimics are labeled.*

3. Methods

3.1 Biochemical essays

3.1.1 Materials. PreScission protease was purchased from Pharmacia. AZT and reagents for enzyme assays were obtained from Sigma. Strain BL21 (Pharmacia) served as expression host. n-MCT and PCV were kindly provided by Dr. Marquez (NIH, Bethesda, USA) and Dr. Johannsen (Forschungszentrum Rossendorf Institute of Bioinorganic and radiopharmaceutical Chemistry research center, Rossendorf, D) respectively. 5-Trifluoromethyl anhydrohexitoluridine (AHTMU), an AHIU analogues, and HBPG were gift of Dr. De Clercq (Rega Institute Katholike Universiteit Leuven, Be) and Dr. Wright (University of Massachussets, USA), respectively. ACV and GCV were purchased by Glaxo-Wellcome and Roche, respectively. dT was obtained from Fluka.

3.1.2 Expression and purification of TK_{HSV1}. TK_{HSV1} was expressed as gluthathion-S-transferase-fusion protein in competent *E. coli* BL21 using the vector pGEX-6P-2-TK (7). The protein was purified by gluthathion-affinity chromatography followed by on-column PreScission protease cleavage using a previously described protocol (7). Purification was monitored by SDS-PAGE and led to a > 90 % pure TK_{HSV1} , which was directly used for k_{cat} determination. Total protein concentration was measured using the Bio-Rad Protein Assay (Bio-Rad).

3.1.3 Spectrophotometric assay for k_{cat} determination. An UV-spectrophotometric test based on a lactate dehydrogenase - pyruvate kinase coupled assay (8) was employed to

monitor ADP formation during substrate phosphorylation. The concentration of ATP and the ligands were 5 mM and 1 mM, respectively. These concentrations are at least five time higher than the binding affinities of the studied compounds allowing the measurement of V_{max} and thus of k_{cat} ($k_{cat} = V_{max}/[E]$). The change in Absorbance at 340 nm was recorded over time and correlate with the k_{cat} of the analyzed substrates for which values were known from the literature and allows the determination of k_{cat} for compounds that are not available in the radiolabelled form (Table 1).

3.2 Quantum chemical calculations

3.2.1 Structural models. In the crystal structure , HSV-1 TK is a homodimeric enzyme with 376 amino acids per subunit. The two subunits are related by C2-symmetry. The enzyme accommodates the nucleobase and sugar-like chain moieties in two different pockets (P1 and P2 in Fig. 2), interacting with the ligand by a specific and extensive H-bond network.

In the pocket P1, the nucleobase moiety (either a thymine or a guanine ring) is stabilized by direct H-bonds with the highly conserved Gln125 and with Arg176 by means of two ordered water molecules. The pyrimidine ring is further fixed by a peculiar interaction with Tyr172 and Met128 (4) in a sandwich-like orientation (9) (10) (Fig. 2).

In P2, the sugar-like chains interacts with the protein via its hydroxyl groups. The 3'OH (and its mimics) forms specific H-bonds with Tyr101 and/or Glu225 (Figs. 2, 3) while 5'OH forms a direct H-bond or water-mediated interactions with Arg163, Glu83 and Arg222 (Fig. 2, 3). In contrast, the polar C1'-O4'-C4' function belonging to dT and the correspondent ether groups of ACV and GCV interact neither with polar or charged

groups of the protein nor with the solvent; instead, they point towards a hydrophobic 1region made up of the Trp88, Ile97, Met128 side chains (Fig. 4).

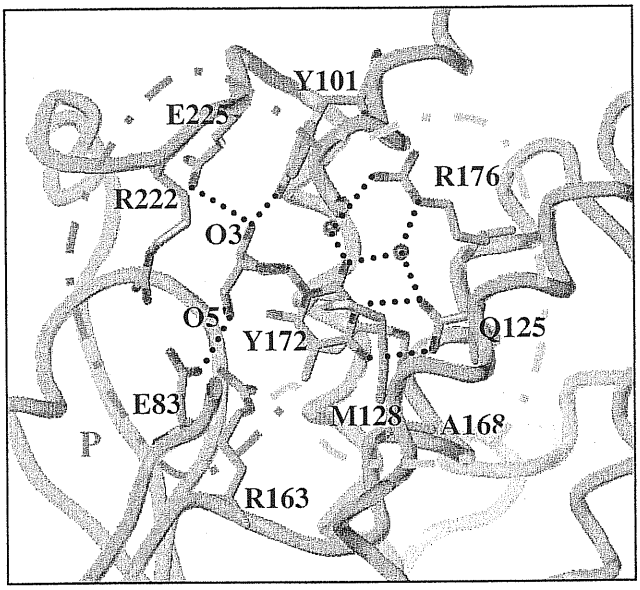


Figure 2. *X-ray structure for the thymidine kinase active site (4). P1 and P2 are the active site portions which accommodate the nucleobase and the sugar like moiety, respectively. Water molecules are shown as red spheres and hydrogen bonds are depicted as dotted line. dT and the amino acids are displayed as capped sticks and are color coded (C: orange; O: red; N: blue; S: yellow). The rest of the protein is shown as ribbon model. The picture was generated with the program SYBYL (Tripos Inc., St. Louis)*

The nucleoside-base ring makes pairwise hydrogen-bond interaction via its 4-carbonyl and 3-NH group with the amine group of highly conserved Gln 125 and is hydrogen bonding with Arg176 by means of two ordered water molecules. Moreover the pirymidine ring of thymidine is fixed between Met128 and Tyr172 forming a sandwiches-like complex. Our structural models for quantum chemical calculation on the sugar moiety of dT-, ACV-, GCV-, PCV-, n-MCT- and HBPG- TK_{HSV1} were constructed

from the correspondent crystal structures (Pdb-entries: 1kim, 2ki5, 1ki2, 1ki3, 1e2k, 1qhi) (5-7). The resolution of the structures ranges from 1.7 to 2.4 Å.

The structure of AHTMU-TK_{HSV1} has not been solved yet, while the structure of the complex AHIU-TK_{HSV1} is known (Pdb entry: 1ki6) (8). AHIU is chemically and structurally extremely similar to AHTMU (Fig.1): the two analogs differ only for the group at position 5 of the nucleobase ring (a iodine in AHIU, a CF_3 in AHTMU). All available structural data show that this substitution does not affect the binding orientation of the sugar mimicking moiety (5). The initial configuration of AHTMU is therefore built from this X-ray structure.

The X-ray structure of the AZT-TK_{HSV1} complex is not available and therefore one has to resort to theoretical structural models. Here we used molecular dynamics based models reported earlier (11).

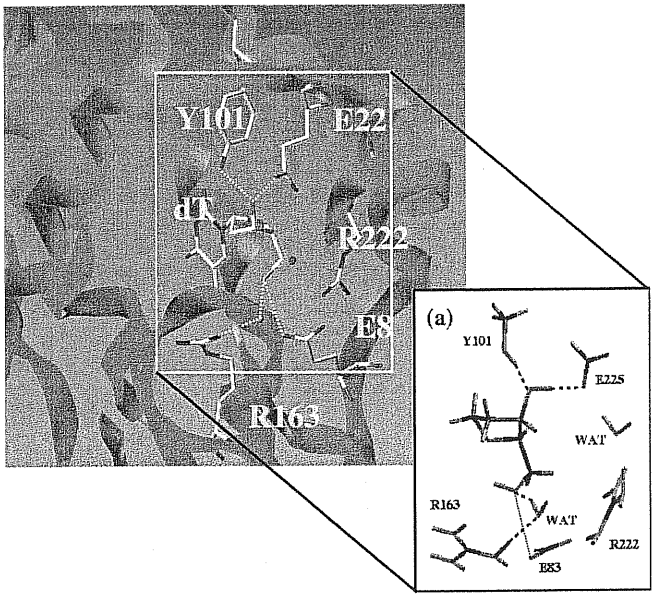


Fig3 Quantum mechanical model for dT/HSV-1 TK complex. Water molecules are represented as red spheres and hydrogen bonds are shown as dotted lines.

The complexes included the sugar-like moieties of the ligands and part of side chains of all the groups directly interacting with it or forming water-mediated hydrogen bonds (Tyr101, Arg163, Glu83 and Glu225, Arg222). Ile97, Met128 side chains, were not included as i) they would have dramatically increased the size of our model complexes; ii) they do not contribute significantly to the electrostatic interactions (the focus of the present work) as they form only weak hydrophobic contacts with the sugar; iii) the position of these groups is essentially the same in all the complexes investigated here (5-7) and therefore their contribution is expected to be rather constant. Hydrogen atoms were added assuming standard bond lengths and bond angles. In Fig. 3 the quantum-mechanical model for dT, the natural substrate, is shown in details.

3.2.2 Quantum Chemistry. Calculations were performed within the framework of density functional theory (DFT) (12) in its Kohn and Sham (13) formulation. In this approach the use of gradient corrections is crucial for correctly describe hydrogen bond interactions. Here we used the prescription of Becke, Lee, Yang and Parr as it has been shown to provide an accurate description of water and hydrogen bonding in biological systems (14-17). The basis set consisted of a plane wave basis set up to a cutoff of 70 Rydberg, the interactions between valence electrons and ionic cores being described by pseudopotentials of the Martins-Troullier type (18). Only the gamma point was used. Our complexes were treated as isolated systems as in ref. (19). Geometry optimization was performed using the direct inversion iterative subspace (DIIS) (20). Cα atoms were constrained to crystallographic position to take into account for the reduced mobility of

residues due to protein environment. A similar procedure has been reported elsewhere. (14)

3.2.3 Electrostatic interaction. Interaction energies between the substrate and the active site are calculated within the central dipole approximation (25) where the dipole is the result of the quantum calculation. Interaction energy was calculated as $E_{charge\text{-}dipole} = (\xi \mu \cdot \mathbf{R})/(4\pi\varepsilon_0 R^3)$, where $\mathbf{R}$ is the distance vector between the centers of charges of the sugar moiety and the residue of charge ξ; μ is the dipole moment for the sugar moiety.
Even if the expansion is limited to large distances between two interacting groups, this model can provide quantitative results for a comparison between systems which have similar geometries. We have reported the energetics in arbitrary units.

3.2.4 Pauling electronegativity. The electronic structure was described with the geometrical analysis of centers of maximally localized Wannier functions (WFC) (21-23). This analysis is useful to investigate the polarity of chemicalbonds, as WFC shifts are related quantitatively to the differences $\Delta\chi$ of Pauling electronegativities (24) with respect to the non interacting compounds (17): an increase of $\Delta\chi$ corresponds to an increase of polarization of the electronic density in the X-Y bond toward the most electronegative atom X.

3.2.5 Protein enviroment effects. In the choice of the model for the active site we included only those residues having a direct H-bond with the substrate. Effects of protein enviroment have been

extimated through a comparison of Wannier-centers shifts for the system in vacuo and in the protein external potential. The procedure was identical to that of ref.(14)

Starting from the crystallographic coordinates for the protein, we added H atoms and a water shell of 8 Å thichness. We performed classical molecular dynamics with *AMBER* for 15 ps at 150 K and other 15 ps at 300K. The electrostatic potential is calculated starting from *AMBER* resp charges for the optimized protein structure and introduced as external potential in the DFT hamiltonian.

4. Results

4.1 Catalytic Activity of TK_{HSV1} .

The catalytic activity of TK_{HSV1} with the compounds listed in Fig. 1 was measured under the same conditions. Table 1 shows that the measured k_{cat} of dT agrees with those reported in literature (26, 27). Also the k_{cat} of ACV corresponds to that measured in two independent laboratories (28, 29). In the literature a value of 0.015 (s^{-1}) is also reported for ACV (26), but in consideration to the new reported values this seems to be an outliner. The k_{cat} values of GCV and PCV are also congruent with the literature values (26, 29). The same agreement has been found for AZT whose k_{cat} value is 0.044 (s^{-1}). The k_{cat} values of the new compounds AHTMU and n-MCT have been reported here for the first time.

Table 1. Values of catalytic activity (k_{cat}) of compounds listed in Fig. 1. The values are the results of at least five independent experiments.

Compound	k_{cat} (s^{-1})[a]	k_{cat} in (s^{-1}) from literature	k_{cat} as rate % of dT[b]
dT	0.348 ± 0.004	0.35[c] – 0.45[d]	100%
ACV	0.115 ± 0.025	0.015[d] - 0.101[e]	27%
GCV	0.100 ± 0.017	0.102[d]	n.d.
PCV	0.045 ± 0.019	n.d	9%
AHTMU	0.210 ± 0.048	n.d	n.d.
n-MCT	0.178 ± 0.010	n.d.	n.d.
AZT	0.044 ± 0.018	0.056[c]	n.d.
HBPG	0	0[f]	n.d.

[a] This work; [b] The rate in % were determined using the substrates at 250 µM (29); [c] in (27); [d] in (26); [e] in (28); [f] HBPG is an inhibitor (30); n.d.: not determined

4.2 Quantum Chemistry Calculations

Overall, the geometry of the complexes are in very good agreement with the experimental structural data as shown by the low root mean squared deviation values between experimental and optimized structures (Table 2). In particular Table 3 shows that the structural parameters of the geometry optimized systems are in good agreement with experimental data. Thus, our first principle approach appears to reproduce very reliably the structural properties of the adducts. Geometry optimization permits also us to establish the H-bond network between the substrate and the enzyme which is crucial in determining the electrostatic properties of the adducts and which is only indirectly deducible from the crystal structure where H are missing.

Table 2. Structure, catalytic activity and electrostatic interactions in ligand-TK_{HSV1} complexes. RMS deviations between X-ray and optimized structure are reported in Å; dipole moments in Debye. Glu225- and Arg163- sugar-like chain interactions are reported in arbitrary units.

Compound	RMS (Å)	k_{cat} (s^{-1})	Dipole(D)	Dipole-GLU225 energy	Dipole-ARG163 energy
DT	0.19	0.348 ± 0.004	1.74	-4.5	-3.1
ACV	0.26	0.115 ± 0.025	1.96	-1.6	-2.2
GCV	0.27	0.100 ± 0.017	1.74	-1.3	-3.4
PCV	0.35	0.045 ± 0.019	1.31	0.0	-0.4
AHTMU	0.26	0.210 ± 0.048	0.40	-1.0	-0.4
n-MCT	0.17	0.178 ± 0.010	2.83	-1.0	-4.9
AZT	0.24	0.044 ± 0.018	3.26	4.9	4.7
HBPG	0.15	0	1.74	0.8	-2.0

4.4.1. Enzyme-sugar interactions. One of the most important physical properties of polar molecules such as ribose and its analogs is given by their electric dipole moment. Fig. 6 offers a visual representation of the calculated dipoles projected to the geometry-optimized structure. The dipole of the sugar moiety of the natural substrate aligns strikingly to the negatively charged Glu225 group. Interestingly, the dipoles of most the prodrugs investigated here also point to this residues. The calculated stabilization energy resulting from the electrostatic interaction is maximum for the natural substrate (Table 3). The dipoles of AZT and the inhibitor HBPG do not point towards the negative residue and as a consequence the interaction energy is repulsive.

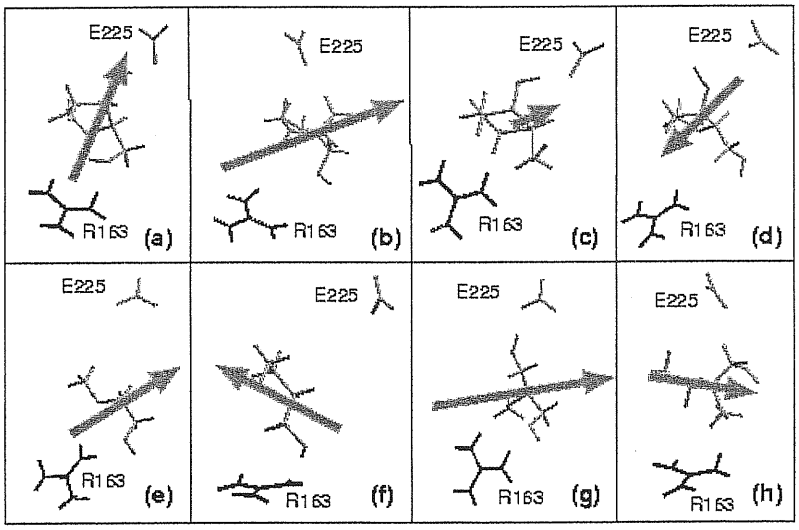


Figure 6. *Electric dipoles of HSV-1 TK sugar like chains. dT (a), n-MCT (b), AHTMU (c), AZT (d), ACV (e), HBPG (f), GCV (g), PCV(h).*

Table 3 dT/TK optimized structure: bond lengths, angles and torsional angles of the sugar moiety. Deviations from the crystallographic structure are shown in brackets.

	Bond length (Å)		Angle (degree)		Torsional angle (degree)
O5-C5	1.48 (0.01)	O5-C5-C4	110 (-2)	O5-C4-C3-C2	56 (6)
C5-C4	1.52 (0.01)	C5-C4-C3	116 (5)	C5-C4-C3-C2	-105 (-14)
C4-C3	1.57 (0.06)	C4-C3-C2	103 (0)	C4-C3-C2-C1	-36 (10)
O3-C3	1.44 (0.04)	O3-C3-C4	110 (6)	C3-C2-C1-O4	46 (9)
C3-C2	1.54 (0.02)	C3-C2-C1	100 (-4)	O3-C3-C2-C1	81 (17)
C2-C1	1.52 (0.04)	C2-C1-O4	104 (8)	C2-C1-O4-C3	-39 (-20)
C1-O4	1.47 (0.01)	C1-O4-C4	106 (-13)	C1-O4-C4-C3	15 (23)
O4-C4	1.48 (0.05)	O4-C4-C3	106 (8)	O4-C4-C3-C2	14 (-17)

In an attempt at correlating electrostatic interactions with kinetics data, we first subdivide the compounds in two major classes. The first includes the compounds bearing a thymine- or a thymine substituted base (namely dT, AHTMU, n-MCT and AZT); the second those bearing guanine (ACV, HBPG, GCV, PCV).

The subdivision is necessary as the computational model did not include the base and consequently the electrostatic calculations do not consider the contributions of the nucleobases (9), whose dipole moments are different but additive [1]. Fig. 7 points out the correlation between the interaction energy dipole-Glu225 and the catalytic constant of the studied ligands appears clearly. The slope of the linear fit depends from the type of sugar mimicking moiety of the ligand. The additivity of the sugar like chains and

[1] The contribution of the nucleobase of the nucleotides to the dipole interaction results additive, even if different for thymine and guanine. Since the chemical bond between the nucleobase and

nucleobase dipoles is confirmed by a rigid shift between the linear fit for the guanine and thymine derivatives (Fig. 7).

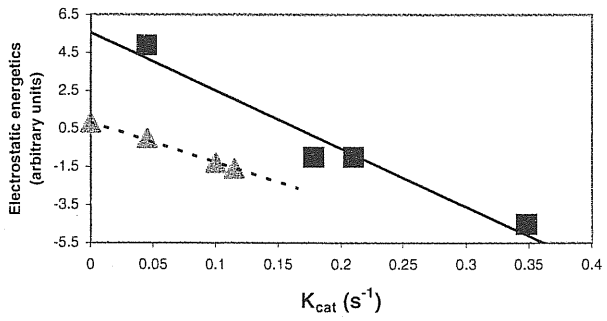


Figure 7. *Correlation between k_{cat} and the sugar-moiety-dipole-Glu225 electrostatic energy (electrostatic energetics). Thymine and guanine derivative are displayed as squares and triangles, respectively. Linear fits are also plotted (R^2 values are 0.954 and 0.994 of thymine and guanine, respectively).*

4.2.2 The role of Arg222, Arg163 and Glu83 interacting groups. Although there is no direct correlation between the k_{cat} and the interaction Arg163-Dipole, the calculated interaction between Arg163 and the sugar is important, consistently with previously published mutagenesis experiments (31). From the crystallographic and mutagenesis studies, Arg222 and Glu83 are essential elements of the kinase machinery, Arg222 forms the anion hole with Arg220 to make the phosphate atom more electrophilic and Glu83 behaves as base on the O5' atom causing an increase in nucleophily. Thus, it is not surprising that Arg222- and Glu83-Dipole interactions appears not to have any direct correlation with k_{cat} (data not shown) being their influence based on a totally different mechanisms.

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the sugar moiety is non-polar we have verified the additivity of the nucleobase and sugar moiety

4.2.3 Sugar/TK_{HSV1} hydrophobic interactions. Our calculations have pointed to the crucial role of the electric dipole moment of the sugar moieties. We now address to the following question: In the natural substrate, is the polar C1'-O4'-C4' (which faces a hydrophobic pocket, Fig 4a) important for the correct orientation of the dipole? To answer this question, we calculate the change of electric dipole associated to replacement of O4' with the CH_2 apolar group (Fig.4b). Our calculation show that the resulting dipole is both smaller and different in orientation relative to the sugar moiety (Fig 4b,c). We conclude that the polar function is essential for a correct alignment of the dipole to E225 charge.

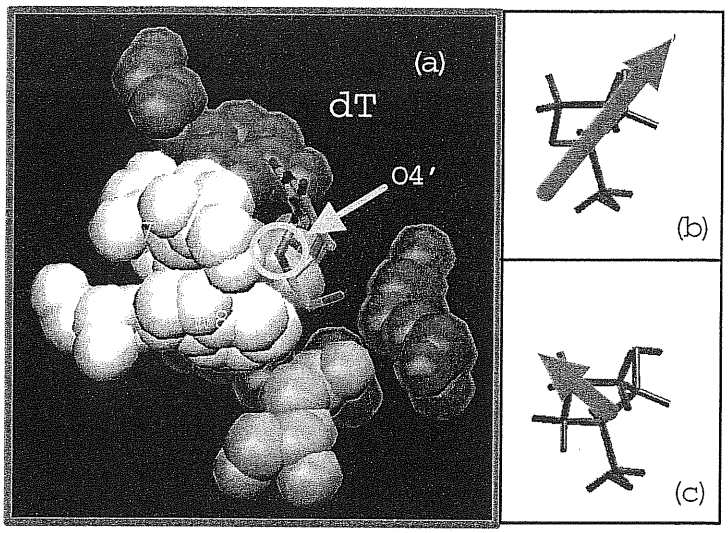

Figure 4. *(a) dT-TK_{HSV1} complex. Orientation of O4' in its hydrophobic pocket; sugar (b) and methylene derivative (c) electric dipoles.*

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dipoles.

4.2.4 Protein environment effects. The field of the protein may be very important for the chemistry of the active site of this and other enzymes (32). Here we estimate the effect of the protein frame by comparing the electronic structure of the complexes in vacuum with those in the presence of the protein. A convenient representation of the electronic structure is given by the Wannier functions, whose centers (WFC) represent chemical concepts such as lone pairs and chemical bonds. Fig. 8 shows that there is no appreciable displacement of the WFC; so that it is possible to conclude that main contribution to the interaction is included in the model we have chosen for quantum mechanics calculations.

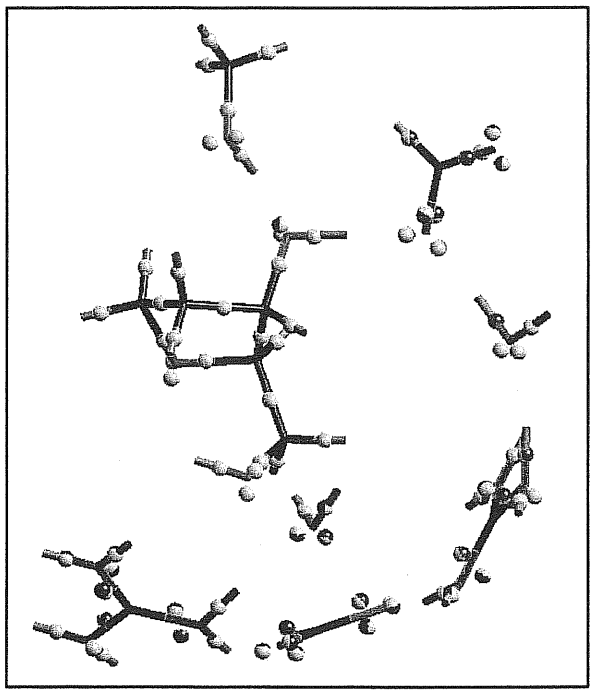

Figure 8. *Electronic structure of dT/HSV-1 TK active site: the Wannier centers (21-23) in the presence and in the absence of the protein electrostatic potential are represented as yellow and green spheres, respectively.*

5. Discussion and Conclusion

Deciphering the binding mode of prodrugs to the enzyme and their catalytic turnover may help to rationally design more and potent prodrugs for antiviral therapy to overcome the problems of resistance or to be used in enzyme-prodrug gene therapy as well as improve variants of TK_{HSV1} for gene therapeutic approaches (33).

The mechanisms ruling binding affinities toward TK_{HSV1} have been previously presented (5, 7, 9, 34). However, (7)the intriguing question posed by the recently published crystal structure of the inhibitor HBPG (6) as to what is the structural basis for the different properties between inhibitor and substrate sharing the same binding mode remained open. This issue prompted us to perform a combined biochemical and quantum-chemical study. Two key parameters have been considered, on the one hand binding affinity and on the other hand catalytic turnover.

Because not all k_{cat} values were available and some conflicting kinetics results had been reported in the literature (Table 1), the catalytic activity of TK_{HSV1} towards all ligands have been measured under the same experimental conditions. In this way, a homogeneous set of measured k_{cat} values have been obtained and compared with the calculations.

The calculated sugar dipole points to Glu225 and the resulting stabilization energy correlates strikingly with the catalytic activity (Fig. 7). These results are consistent with a previously published study on E225L TK_{HSV1}. Indeed this mutant exhibits a loss in

binding affinity proportional to the loss of one hydrogen bond and more interesting a dramatic drop in catalytic activity (27).

Glu225 contributes to the binding affinity of the prodrug by H-bond interactions with the 3'OH group (4, 5, 7, 35, 36). Our calculations point to its important role in catalysis derived from its interaction with the dipole. In contrast no correlation have been found between the calculated terms and binding affinity expressed as K_i or K_M.

This type of calculations are very straight forward and can be extended in the future to new compounds.

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In conclusion we indicated a substrate-enzyme molecular interaction which shows to be relevant to catalysis. We have shown a nice correlation between dipole-charge

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5. Discussion and Conclusion

Deciphering the binding mode of prodrugs to the enzyme and their catalytic turnover may help to rationally design more and potent prodrugs for antiviral therapy to overcome the problems of resistance or to be used in enzyme-prodrug gene therapy as well as improve variants of TK_{HSV1} for gene therapeutic approaches (33).

The mechanisms ruling binding affinities toward TK_{HSV1} have been previously presented (5, 7, 9, 34). However, (7)the intriguing question posed by the recently published crystal structure of the inhibitor HBPG (6) as to what is the structural basis for the different properties between inhibitor and substrate sharing the same binding mode remained open. This issue prompted us to perform a combined biochemical and quantum-chemical study. Two key parameters have been considered, on the one hand binding affinity and on the other hand catalytic turnover.

Because not all k_{cat} values were available and some conflicting kinetics results had been reported in the literature (Table 1), the catalytic activity of TK_{HSV1} towards all ligands have been measured under the same experimental conditions. In this way, a homogeneous set of measured k_{cat} values have been obtained and compared with the calculations.

The calculated sugar dipole points to Glu225 and the resulting stabilization energy correlates strikingly with the catalytic activity (Fig. 7). These results are consistent with a previously published study on E225L TK_{HSV1}. Indeed this mutant exhibits a loss in

binding affinity proportional to the loss of one hydrogen bond and more interesting a dramatic drop in catalytic activity (27).

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