Effect on substrate binding of an alteration at the conserved aspartic acid-162 in herpes simplex virus type 1 thymidine kinase

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Despite the extensive use of antiviral drugs for the treatment of herpesvirus infections and as prodrugs for ablative gene therapy of cancer, little structural information about the drug activating enzyme, herpes simplex virus type 1 thymidine kinase (TK), was available until recently. In the absence of the three-dimensional structure we sought to elucidate the function of the key aspartic acid residue (D162) present within a highly conserved tripeptide motif that is thought to function in nucleoside binding. In this study we generated a mutant, D162Q, by site-directed mutagenesis, purified both the wild-type and mutant TKs to near homogeneity by single-step affinity chromatography and determined the kinetic parameters for thymidine, ATP, dTMP and dTTP interactions. A 12-fold increase in $K_m$ for thymidine by D162Q TK ($K_m = 6.67 \mu M$) relative to wild-type enzyme ($K_m = 0.56 \mu M$) was observed and the absence of any alteration in $K_m$ for ATP suggests that D162 participates in nucleoside binding. Furthermore, the $K_i$ for dTMP is significantly higher for D162Q TK than for HSV-1 TK which is indicative of a shared or overlapping binding site with thymidine. This assessment is further supported by the different inhibition patterns of D162Q and wild-type TKs observed using [$\gamma$-32P]5-N2dUMP photoaffinity labeling in the presence of thymidine, ganciclovir or dTMP. Interestingly, the $K_i$ for dTTP was 30-fold lower for D162Q TK ($K_i = 2.2 \mu M$) than for the wild-type enzyme ($K_i = 65.8 \mu M$) which provides further evidence of the importance of D162 in TK function.

Introduction

Thymidine kinase (TK; EC 2.7.1.21) is a key enzyme in the salvage pathway of dTTP metabolism, catalysing the transfer of the $\gamma$-phosphate of ATP to thymidine to produce dTMP. The TKs encoded by the virus family Herpesviridae are unique in that they possess additional enzymatic activities and low substrate specificities. Perhaps the most thoroughly studied TK is that of herpes simplex virus type 1 (HSV-1), which has been the target for the development of antiviral drugs such as acyclovir (ACV) and ganciclovir (GCV). Despite the more recent use of HSV-1 TK as a suicide enzyme in gene therapy of cancer, until very recently the only clues to residues that might participate in substrate binding or catalysis came from mutant TKs isolated from drug resistant viruses (Larder et al., 1983; Darby et al., 1986) and from alignment studies (Balasubramaniam et al., 1990; Gentry, 1992; Koonin & Senkevich, 1992). In the past few years, several mutational studies have focused on one region of the enzyme, spanning residues 155 to 177 (Munir et al., 1992; Black & Loeb, 1993). These findings have led to the elucidation of important residues within this region although detailed kinetic evaluation of the majority of mutants generated has not been reported. From one study a particular residue appears key – an aspartic acid residue at position 162 (D162) (Black & Loeb, 1993). Interestingly, from an alignment of the HSV-1 and monkeypox virus TK primary amino acid sequences, Folkers & Trumpp (1987) suggested that the difference in substrate specificities between the Herpesviridae and non-herpesvirus TKs might be due to the aspartic acid (D162) in HSV-1 TK and a glutamine (Q114) in monkeypox virus TK. Black & Hruby (1992) demonstrated that an alteration of Q114 to aspartic acid (Q114D) in vaccinia virus TK did not lead to a broader...
substrate specificity but rather resulted in a dramatic reduction in feedback inhibition by dTTP. Because these enzymes differ radically from each other on many levels (monomer size, subunit number, level of feedback inhibition, substrate specificity) it is unclear whether corresponding residues participate in similar activities. While recent X-ray diffraction structural studies place D162 within the active site (Wild et al., 1995; Brown et al., 1995), how D162 participates in substrate interactions is not obvious. In the present study, we examine the role of D162 in substrate binding and dTTP inhibition both kinetically and with photoaffinity probes using a site-directed mutant HSV-1 TK (D162Q). Such information may assist in the development of novel antiviral drugs that require activation by TKs of HSV-1 and related viruses.

**Methods**

**Bacterial strains.** E. coli Cpl26 [F' cat (+ = pC105 M13* Cm'^')/dut ung-1 thi-1 relA1 spoT1 merA] was used to produce the uracil-containing single-stranded DNA template for site-directed mutagenesis. E. coli BL21(DE3) [F ompT hsdSB (r B rnB) with DE3, a lambda prophage carrying the T7 RNA polymerase gene] was used to produce wild-type and mutant TKs for purification (Novagen).

**Materials.** Substrate [methyl-3H]thymidine (specific activity 87 Ci/mmole) for TK assays was purchased from Amersham. Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs. All other reagents were obtained from Sigma except where designated.

**Site-directed mutagenesis.** The single-stranded DNA template for site-directed mutagenesis was isolated from pT7:HSVTK (Cpl26) (Black & Hruby, 1992) as described in Black & Hruby (1990). The 18-mer mutagenic oligonucleotide (5'GGGATGGCTTGGAAGATG3') was purchased from Operon, Alameda, Calif., USA. After confirmation of the mutation by sequencing, both the wild-type and D162Q mutant Ncol fragments were independently subcloned into the Ncol site of pET8c (Studier et al., 1990) and the orientation and sequence confirmed. The plasmids, pET8c:D162Q and pET8c:HSVTK, were then used to transform E. coli BL21(DE3) for TK protein overexpression. Plasmid-containing strains were grown on 2 X YT containing carbenicillin at 50 µg/ml at 37 °C.

**Protein induction and affinity purification.** Overnight cultures of pET8c:HSVTK and pET8c:D162Q [BL21(DE3)] were used to inoculate 1 litre of M928 containing carbenicillin at 20 µg/ml (Studier et al., 1990). At an OD600 of 0.1, IPTG was added to a final concentration of 0.4 mM and the cultures allowed to grow for an additional 3 h. The cultures were then chilled on ice for 20 min and the cell pellets collected by centrifugation. The pellets were washed once in 25 ml of cold cell wash buffer (50 mM-Tris, pH 7.5, 5 mM-EDTA, 10% sucrose) and placed at -70°C overnight. The pellets were resuspended in 12 ml of Buffer 1 (50 mM-Tris, pH 7.5, 5 mM-EDTA, 2 mM-DTT, 10% sucrose, 0.1 mM-PMSF) and split into two 10 ml Oakridge centrifuge tubes; 1 ml of Buffer 1 containing 3 mg lysozyme was added to each tube. After 1 h on ice the tubes were incubated at 37°C for 3 min and 1 ml of Buffer 1 containing protease inhibitor mix (62.5 mg aprotinin, 1:25 mg leupeptin, 1:25 mg pepstatin per ml) was added. A cleared lysate was formed by centrifugation in a 50 Ti rotor at 35000 r.p.m. for 30 min at 4°C. The lysate was dispensed and stored at -70°C.

Purification of wild-type and mutant TKs was performed by affinity chromatography on Sepharose CL-4B (Pharmacia) coupled to p-amino-phenylthymidine 3'-phosphate (Kowal & Markus, 1976; Lee & Cheng, 1976). All chromatography steps were done at 4°C. The 1 ml bed volume column was prepared by passing 10 ml of Buffer 1 followed by 10 ml of Absorption Buffer (50 mM-Tris, pH 7.5, 2 mM-DTT, 10% sucrose, 25 mM-magnesium acetate, 10 mM-ATP) over the column. Cleared bacterial lysates were diluted 1:1 in Absorption Buffer (2 ml each) and passed over the column three times. The column was washed with 3 ml aliquots of Adsorption Buffer (W1-W3). TK protein was eluted in three 1 ml aliquots of Thymidine Buffer (0.5 mM-Tris, pH 7.5, 2 mM-DTT, 10% sucrose, 50 mM-KCl, 0.5 mM-thymidine) (E1-E3). The column was reactivated with 10 ml of High Salt Buffer (50 mM-Tris, pH 7.5, 2 mM-DTT, 10% sucrose, 0.5 mM-KCl) followed by 10 ml 50 mM-Tris, pH 7.5. The column was stored in 50 mM-Tris, pH 7.5 containing 0.004% sodium azide at 4°C. Fractions E1-E3 were dialysed against four 1 litre changes of 50 mM-Tris, pH 7.5, 2 mM-DTT, 10% sucrose at 4°C. Both HSV-1 and D162Q TK purifications appeared >95% pure as estimated from Coomassie Blue stained denaturing protein gels. Protein concentrations were determined using the Bio-Rad reagent according to the manufacturer’s instructions. BSA was used as the protein standard.

**Photoaffinity labelling.** Induction for overexpression of HSV-1 and D162Q TKs was as above. Protein purification of greater quantities of HSV-1 and D162Q TKs for photoaffinity labelling was as described in Rechtin et al. (1995). Briefly, cleared bacterial lysates were prepared by centrifugation at 10000 g for 30 min and HSV-1 TK was partially purified by DEAE-cellulose chromatography. Synthesis of [α-32P]N9-dUMP and [α-32P]8-N9-ATP and the photolabelling reactions were as described in Rechtin et al. (1995). For competition experiments, different concentrations of nonradio-labelled competitors were incubated in the reaction mixture for 20 s prior to the addition of photoprobe (15 µM-[^3]H]N9-dCMP or [^3]H]8-N9-ATP).

**Enzyme assays.** TK assays were as described in Hruby & Ball (1981) except that all assays were performed at 37°C and washes were done at room temperature. A standard assay reaction contains 1 µM-[methyl-3H]thymidine (87 Ci/mmole), 100 mM-sodium phosphate, pH 6, 10 mM-ATP and 25 µM-magnesium acetate. All reactions were performed in triplicate at least three times. The Michaelis constant (Km) for thymidine and the km (Vmax/EI) values were determined with the use of double reciprocal plots. The kinetic parameter (Kc) for ATP was obtained by varying the ATP concentration with a saturating (67%) concentration of [methyl-3H]thymidine fixed at approximately twice the Kc value for each enzyme (1 µM for wild-type TK and 10 µM for D162Q). For assays to examine the magnesium requirement, assay mixes contained 1 µM-[methyl-3H]thymidine for both wild-type and mutant except that cold thymidine was added to a final reaction concentration of 10 µM for D162Q. The Kc values for dTTP and dTMP with thymidine as substrate were determined from double reciprocal plots (Segel, 1976).

**Results and Discussion**

**Alignment of herpesvirus and non-herpesvirus TKs**

In general, *Herpesviridae* and non-herpesvirus TKs share little primary amino acid sequence identity. One conserved region in both types of TK has been postulated to be involved in substrate binding; Site 3 in herpesvirus TKs (residues 162 to 164) (Balasubramaniam et al., 1990) and Site IV in non-herpesvirus TKs kinases (residues 104 to 115 of vaccinia virus TK (Black & Hruby, 1990) and later redefined to comprise...
residues 113 to 117) (Folkers & Trumpp, 1987; Black & Hruby, 1992). A consensus sequence has been generated from comparisons of 12 Herpesviridae TKs and 12 non-herpesvirus TKs including enzymes from human, mouse, chicken and members of the poxvirus family (Black & Hruby, 1992). The primary amino acid sequences at Sites 3 and IV are very similar except for the position corresponding to the completely conserved aspartic acid at position 162 (HSV-1 position) in herpesvirus TKs. In the non-herpesvirus TKs, a glutamine residue is present in 8 of the 12 members of this group and a hydrophilic residue occurs in 9 of the 12 members. Folkers & Trumpp (1987) suggested that the aspartic acid vs. glutamine residue results in the difference in substrate specificity between these two TK groups. Herpesviridae TKs have a low substrate specificity and phosphorylate not only thymidine analogues but also guanosine analogues (ganciclovir and acyclovir) as well as deoxycytidin and thymidylate (Cheng, 1976; Elion et al., 1977). The non-herpesvirus TKs primarily phosphorylate thymidine and very closely related analogues such as azidothymidine (AZT) (Munch-Petersen et al., 1991). Perhaps the presence of an uncharged, hydrophilic residue at that position restricts substrate utilization. Mutational analysis of glutamine at position 114 in vaccinia virus and experimental results from studies with these mutants demonstrated no alteration in substrate affinity although a dramatic reduction in feedback inhibition by dTTP was observed (Black & Hruby, 1992). While a glutamine or hydrophilic residue appears not to be essential for vaccinia virus TK activity, the aspartic acid (or other negatively charged residue) at position 162 in HSV-1 TK may be. In support of this, Fetzer et al. (1993) concluded that an HSV-1 TK mutant, D162N, lacks enzymatic activity as they were unable to recover HSV-1 TK D162N-recombinant vaccinia viruses in HAT selection medium.

Sequence analysis of a limited number of active HSV-1 TK clones generated by random sequence mutagenesis and selected by positive genetic complementation revealed that D162 was only substituted by another negatively charged residue, glutamic acid, or glycine. The glycine substitution exhibited barely detectable activity in vitro while the D162E mutant displayed thymidine phosphorylation, albeit at a reduced rate compared to the wild-type enzyme. From their studies, Black & Loeb (1993) suggested that a negatively charged residue at position 162 might be essential for activity. This compelled us to examine further the role of D162 in HSV-1 TK by generating and characterizing the site-specific mutant D162Q.

**Mutant and wild-type TK protein purification**

A one-step affinity purification of the wild-type HSV-1 and mutant D162Q TKs yielded proteins that were near homogeneity as estimated from Coomassie stained SDS containing polyacrylamide gels (Fig. 1). Gel electrophoresis of D162Q purification fractions was indistinguishable from that of HSV-1 TK (data not shown). The concentration of purified TK proteins was 0.016 µg/µl and 0.01 µg/µl for HSV-1 TK and D162Q, respectively. The level of background activity from the endogenous TK that might be co-purified with the herpesvirus TK was determined on fractions from a mock purification (vector plasmid, no HSV-1 TK gene). These
Table 1. Kinetic parameters of mutant and wild-type TKs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>K_m (µM)</th>
<th>k_{cat} (s^{-1})</th>
<th>k_{cat}/K_m (s^{-1} µM^{-1})</th>
<th>K_m (µM)</th>
<th>K_i (µM)</th>
<th>K_f (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 TK</td>
<td>0.56</td>
<td>0.212</td>
<td>0.38</td>
<td>32.3</td>
<td>12.5</td>
<td>65.8</td>
</tr>
<tr>
<td>D162Q</td>
<td>6.67</td>
<td>0.0092</td>
<td>0.0014</td>
<td>35.7</td>
<td>51.6</td>
<td>2.2</td>
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<tr>
<td>Fold difference</td>
<td>12</td>
<td>23</td>
<td>271</td>
<td>1.1</td>
<td>4.1</td>
<td>30</td>
</tr>
</tbody>
</table>

* The k_{cat} values were calculated using the equation V_{max} = k_{cat} * [E] where E = the total enzyme concentration and is based on one active site/dimer. Assay conditions are described in Methods.

Thymidine/thymidylate binding site

From alignment and mutational studies, the highly conserved D162 was proposed to participate in nucleoside binding (Balasubramaniam et al., 1990; Black & Loeb, 1993). In order to examine experimentally the role of D162 in substrate binding, the kinetic parameters of purified wild-type and mutant (D162Q) TKs were determined. Both enzymes displayed Michaelis–Menten kinetics throughout the concentration range studied (0.05 to 1 µM-thymidine for wild-type TK and 0.25 to 10 µM-thymidine for D162Q) and no substrate inhibition or activation was observed. The K_m values were determined from Lineweaver–Burk plots. The Michaelis constant (K_m) for HSV-1 TK was 0.56 µM which is in agreement with previously reported values (Munir et al., 1992). D162Q demonstrated a 12-fold increase in K_m for thymidine (K_m = 6.67 µM) compared to the wild-type HSV-1 TK (Table 1). The large difference in K_m between the mutant and wild-type enzymes indicates a marked reduction in the ability of D162Q to bind thymidine and supports the proposed role of D162 in substrate binding.

Fractions contained less than 0.01% wild-type HSV-1 TK activity (data not shown).

Not only is substrate binding affected in the mutant but the turnover number (k_{cat}) is significantly reduced, resulting in a very large difference in the specificity constant or k_{cat}/K_m (271-fold) with respect to the wild-type enzyme. A decrease in k_{cat} for thymidine in D162Q (Table 1) may reflect minor, local structural perturbations caused by the substituted residue which interferes with phosphoryl transfer and/or product release.

To evaluate further the participation of D162 in substrate binding we used an active site directed photoaffinity analogue of dTMP, 5-N3dUMP (Rechtin et al., 1995). Differential competition of [32P]5-N3dUMP photoincorporation by nucleosides and nucleotides was then used to identify alterations at the active site. Previous photoaffinity labelling studies of wild-type HSV-1 TK and an acyclovir resistant mutant, C336Y, indicated specific active site photoincorporation of 5-N3dUMP with saturation and competition results consistent with K_m values for thymidine, thymidylate and ATP (Rechtin et al., 1995). As shown in Fig. 2 (a) photoincorporation of [32P]5-N3dUMP into HSV-1 TK was saturable (half-maximal: 6 µM), while saturation of photolabel insertion into the D162Q mutant was not observed at concentrations up to 30 µM. This difference may be indicative of perturbations within the dTMP binding site. Competition of 5-N3dUMP photoincorporation was
examined using several compounds including thymidine, thymidylate, ATP and ganciclovir. The concentration of competitor that inhibited photolabelling by 50% (ICPs0) is shown in Table 2. Except for ATP, all the compounds tested inhibit D162Q photolabelling significantly less than HSV-1 TK. The higher ICPs0 of [32P]5-N3dUMP photolabelling of D162Q by thymidine and thymidylate suggests that both the thymidine and thymidylate binding sites have been altered.

As might be expected with a shared or overlapping site, the K i for thymidylate using thymidine as the substrate was also altered in D162Q (K i = 51.6 μM). The K i (dTMP) value determined for the wild-type enzyme (K i = 12.5 μM) is in agreement with the published value of 13 μM (Table 1) (Chen et al., 1979). Inhibition of thymidine phosphorylation for both wild-type and Dlb2Q enzymes by dTMP was competitive (data not shown). Taken together, the kinetic and photoaffinity labelling data presented here are consistent with a shared or overlapping thymidine and thymidylate binding site. Furthermore, the observed differences in K m (thymidine), K i (thymidylate) and ICPs0 values (5-N3dUMP) between wild-type and D162Q enzymes indicate that position 162 participates in nucleoside binding.

### Mg2+/ATP binding

To evaluate the possibility that the introduction of this mutation results in a change in the affinity for the phosphate donor ATP, the K m for ATP was determined. Similar K m values for both wild-type and mutant enzymes were observed (Table 1). The requirement for a divalent cation (Mg2+) was also unaltered (data not shown).

To characterize further the effect on ATP binding of the aspartic acid to glutamine mutation, a photoaffinity analogue of ATP, [γ-32P]8-N3ATP, was used. Saturation of photo-incorporation curves for HSV-1 and D162Q TKs were similar with half-maximal saturation at 90 μM (HSV-1 TK) and 115 μM (D162Q) (Fig. 2b). The ICPs0 values determined for the inhibition of [32P]8-N3ATP photolabelling by ATP, GTP, TTP and CTP are shown in Table 3: little difference was observed for ATP inhibition of D162Q as compared to the wild-type enzyme. The kinetics of saturation and competition for [32P]8-N3ATP are similar between the two enzymes indicating that the D162Q nucleotide (ATP) binding site is not altered and maintains wild-type-like properties. This is consistent with previous observations that 8-N3ATP and 5-N3dUMP bind at different sites (Rechtin et al., 1995) and is supported here by the similarities in ATP competition for [32P]5-N3dUMP binding between wild-type and D162Q enzymes (Table 2). Most likely ATP binds to a canonical ATP binding site consensus sequence (residues 56 to 63) that has been functionally identified by kinetic analysis of site-directed mutants within that region (Liu & Summers, 1988). The kinetic and photolabelling studies presented here indicate that only the interaction between nucleoside and enzyme is affected in D162Q and not the enzyme interactions with the phosphate donor or divalent cation.

### dTTP interaction

Because the Q114D mutation in vaccinia virus TK resulted in an apparent abolition of feedback inhibition by dTTP (Black & Hruby, 1992), the inhibitory effect of dTTP on the reciprocal aspartic acid to glutamine mutation in HSV-1 TK (D162Q) was examined. Interestingly, kinetic analysis of D162Q shows a 30-fold reduction in K i with dTTP as inhibitor (K i = 2.2 μM) compared to the wild-type K i value (K i = 65.8 μM) (Table 1). Furthermore, wild-type and D162Q inhibition by dTTP was competitive (data not shown) as had previously been reported for the wild-type enzyme (Chen et al., 1979). This suggests that dTTP binds at the same site or at a site overlapping the thymidine/thymidylate binding site. Alternatively, dTTP may bind at a distinct ‘regulatory’ site, which causes a conformational change in the enzyme that masks or distorts the substrate binding site, i.e. by feedback inhibition (Chen et al., 1979; Segel, 1976). It is plausible that dTTP binding occurs in

### Table 2. Inhibition of [γ-32P]5-N3dUMP photolabelling of wild-type and D162Q HSV-1 TKs

<table>
<thead>
<tr>
<th>Compound</th>
<th>HSV-1 TK (ICPs0)</th>
<th>D162Q (ICPs0)</th>
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</thead>
<tbody>
<tr>
<td>Thymidine</td>
<td>1.5</td>
<td>34</td>
</tr>
<tr>
<td>dTMP</td>
<td>12.5</td>
<td>146</td>
</tr>
<tr>
<td>ATP</td>
<td>150</td>
<td>175</td>
</tr>
<tr>
<td>GCV</td>
<td>100</td>
<td>&gt; 300</td>
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</table>

### Table 3. Inhibition of [γ-32P]8-N3ATP photolabelling of wild-type and D162Q HSV-1 TKs

<table>
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<tr>
<th>Compound</th>
<th>HSV-1 TK (ICPs0)</th>
<th>D162Q (ICPs0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>80</td>
<td>88</td>
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<tr>
<td>GTP</td>
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<td>240</td>
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</tr>
<tr>
<td>CTP</td>
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<td>&gt; 600</td>
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</table>
a way that is facilitated by minor perturbations manifested by the glutamine substitution within the nucleoside binding site. The lack of a negatively charged residue at position 162 may allow dTTP to access and inhibit the enzyme in a way that is hindered in the wild-type enzyme.

Alteration of the vaccinia virus TK at position 114 in Site IV to an aspartic acid (Q114D) results in a loss of dTTP inhibition (Black & Hruby, 1992). An aspartic acid to glutamine change in HSV-1 TK at position 162 (D162Q) results in a 30-fold increase in sensitivity to dTTP inhibition (Table 1). Whether the differences in dTTP inhibition observed with the vaccinia virus Q114D TK and the HSV-1 D162Q TK mutant relative to their respective wild-types reflects a similar role for these residues or sites in enzyme mechanism remains to be determined. Nonetheless, it is intriguing that similar (reciprocal) mutations in generally non-homologous enzymes can cause commutable changes in the same function (dTTP inhibition).

Conclusion

In the absence of any structural information on HSV-1 TK at the initiation of these studies, we sought to determine the role in substrate binding of the key aspartic acid residue within the highly conserved Site 3 motif of Herpesviridae TKs. Observed differences in inhibition patterns of photoaffinity labelling are consistent with Km differences between wild-type and D162Q TKs. There is a dramatic reduction in the ability of all compounds examined, with the exception of ATP, to inhibit photoaffinity labelling in the putative nucleoside binding site of the wild-type enzyme. Furthermore, our results using thymidylate as a competitor of 5-N3dUMP centre of the herpes simplex virus thymidine kinase involves an interaction between three distinct regions of the polypeptide. These results indicate that D162 participates in nucleoside binding but is not involved per se in ATP or Mg2+ interactions. The enhanced inhibition displayed by D162Q in the presence of dTTP indicates that position 162 plays an important role in dTTP binding.

Only very recently, two reports on the X-ray diffraction structure of HSV-1 TK were published (Wild et al., 1995; Brown et al., 1995). In agreement with the data presented here, Wild et al. (1995) and Brown et al. (1995) place the Site 3 motif (DRH) in the active site, in close proximity to the substrate. While X-ray structure predictions reveal snapshots of enzyme-substrate interactions the study reported here provides experimental support that the conserved D162 participates in binding thymidine and nucleoside analogues, thymidylate and probably the end-point inhibitor, dTTP. We anticipate that the identification of residues involved in substrate interaction of target molecules such as HSV-1 TK residue D162 will aid in the rational design of novel anti-herpetic drugs.

References


Cheng, Y.-C. (1976). Deoxythymidine kinase induced in HeLa tk- cells by herpes simplex virus type 1 and type II: substrate specificity and kinetic behavior. Biochimica et Biophysica Acta 452, 370–381.


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