Mutations Distal to the Substrate Site Can Affect Varicella Zoster Virus Thymidine Kinase Activity: Implications for Drug Design

Kamel El Omari, Sandra Liekens, Louise E. Bird, Jan Balzarini, and David K. Stammers

Division of Structural Biology, The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom (K.E.O., L.E.B., D.K.S.); and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium (S.L., J.B.)

ABSTRACT

Varicella zoster virus encodes a thymidine kinase responsible for the activation of antiviral nucleoside prodrugs such as acyclovir. In addition, herpes virus thymidine kinases are being explored in gene/chemotherapy strategies aimed at developing novel antitumor therapies. To investigate and improve compound selectivity, we report here structure-based site-directed mutagenesis studies of varicella zoster virus thymidine kinase (VZVTK). Earlier reports showed that mutating residues at the core of the VZVTK active site invariably destroyed activity; hence, we targeted more distal residues. Based on the VZVTK crystal structure, we constructed six mutants (E59S, R84V, H97Y/A, and Y21H/E) and tested substrate activity and competitive inhibition for several compound series. All VZVTK mutants tested retained significant phosphorylation activity with dThd as substrate, apart from Y21E (350-fold diminution in the kcat/Km). Some mutations give slightly improved affinities: bicyclic nucleoside analogs (BCNAs) with a p-alkyl-substituted phenyl group seem to require aromatic ring stacking interactions with residue 97 for optimal inhibitory effect. Mutation Y21E decreased the IC50 value for the BCNA 3-(2'-deoxy-β-D-ribofuranosyl)-6-octyl-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (Cf1368) 4-fold, whereas mutation Y21H increased the IC50 value by more than 15-fold. These results suggest that residue 21 is important for BCNA selectivity and might explain why HSV1TK is unable to bind BCNAs. Other mutants, such as the E59S and R84V thymidine kinases, which in wild-type VZVTK stabilize the dimer interface, give opposite results regarding the level of sensitivity to BCNAs. The work described here shows that distal mutations that affect the VZVTK active-site may help in the design of more selective substrates for gene suicide therapy or as anti-varicella zoster virus drugs.
thymidine. In addition, VZVTK and HSV1TK have thymidine kinase activity, which converts thymidine monophosphate to thymidine diphosphate. Thereafter, thymidine diphosphate is converted to thymidine triphosphate by cellular kinases. Compared with mammalian thymidine kinases, viral thymidine kinases have a broader substrate specificity and can phosphorylate many nucleoside analogs, including purine derivatives. This variation in substrate specificity is the basis for the action of nucleoside analogs as antiviral drugs and can be attributed to differences in the active sites of the viral compared with host thymidine kinases.

The interest in the potential of suicide genes and in antiviral drugs has encouraged studies of viral thymidine kinases and their ligands. Each thymidine kinase shows different affinities for various nucleoside analogs (De Clercq, 2004). Knowing the structure of these enzymes and their active site architecture can assist in the design of improved drugs and, in the case of gene suicide therapy, can help to direct their engineering by site-directed mutagenesis for improved or more selective thymidine kinases.

We have reported the crystal structure of VZVTK in a product complex with BVDU monophosphate and ADP (Bird et al., 2003), which was used as the basis for the current mutagenesis studies. The previously reported site-directed mutagenesis of residues making direct contact with the thymidine pyrimidine ring invariably led to almost complete loss of enzyme activity, even when substituting HSV1TK residues into VZVTK (Roberts et al., 1991; Suzutani et al., 1993). Because it was necessary to retain significant enzyme activity, we tried a more subtle approach. Analysis of the VZVTK structure suggested that the mutation of some residues more distal to the active site may indirectly affect substrate binding. At the VZVTK dimer interface, a loop is stabilized by hydrogen bonds (Fig. 3). These interactions are absent in HSV1TK. The hydrogen-bonded residues are Glu59, His88, and Arg84 (Glu59 and His88 belong to chain A and Arg84 to chain B). Without such stabilizing interactions, the loop conformation is thus altered in the HSV1TK structure, and it is responsible for different positioning of Tyr66 and Gln95 in the active site. We decided to mutate these residues to their counterparts in HSV1TK, namely E59S and R84V, with the expectation that they could indirectly affect the conformation of the active site by introducing more flexibility. Because certain molecules like ganciclovir have much higher affinity for HSV1TK than for VZVTK, we also decided to mutate residues His97 and Tyr21 to the corresponding HSV1TK residues, respectively, Tyr132 and His58 (Fig. 2). His97 is positioned closer to the bromo-vinyl substituent of BVDU.

Fig. 1. Structural formulae of nucleoside analogs.
than would be the HSV1TK homolog Tyr132, whereas Tyr21 makes weaker van der Waals contacts with the ribose sugar compared with His58.

This mutational analysis has revealed how particular residues may contribute to the binding of nucleoside analogs such as the BCNAs. Such information should be of value for the rational design of new antitherapeutics and for engineering more selective thymidine kinases for use in gene therapy for tumors.

Materials and Methods

Cloning. The plasmid pGEX6P1-VZVTK, described previously (Bird et al., 2003) was used to generate six mutants of VZVTK (R84V, E59S, H97Y, H97A, Y21H, and Y21E) using the QuickChange mutagenesis kit from Stratagene (La Jolla, CA). The presence of mutations was checked by DNA sequencing.

Expression and Purification. The expression plasmid pGEX6P1-VZVTK was transformed into Rosetta(DE3)plysS cells for protein expression. Cultures were grown at 37°C in Luria broth medium supplemented with 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol. When the A600 reached 0.7, the cells were induced with 1 mM isopropyl-β-D-thiogalactopyranoside and incubated for an additional 16 h at 25°C. Cells were harvested by centrifugation and resuspended in buffer A (50 mM Tris- HCl, pH 7.4, 200 mM NaCl, 1 mM dithiothreitol, and 2% sarkosyl) + 1 mM phenylmethylsulfonyl fluoride and then disrupted by sonication. The supernatant was clarified by centrifugation and applied to a 5-ml glutathione-Sepharose column (GST-trap column; GE Healthcare, Little Chalfont, Buckinghamshire, UK) pre-equilibrated with buffer A. After the baseline had returned to zero, 0.5 mg of GST-3C protease in 5 ml of buffer A was added to the column, which was then incubated overnight at 4°C. A 1-ml glutathione-Sepharose column was linked to the outlet of the 5-ml column to trap any GST-VZVTK, GST, or GST-3C protease that did not stay bound to the first column during elution with buffer A. The eluted VZVTK was concentrated to 2 mg/ml. The same purification procedures were used for all the mutants.

Radiochemical. The radiolabeled [CH3-3H]dThd (specific radioactivity, 83 Ci/mmol) was obtained from GE Healthcare. Radiochemical.

Compounds. The origin of the test compounds (Fig. 1) was as follows: BVDU was from Searle (Pareham, Hampshire, UK); IVDU was synthesized by P. Herdewijn at the Rega Institute for Medical Research (Katholieke Universiteit Leuven, Leuven, Belgium); BVaraU was provided by H. Machida (Yamasa Shoyu Co., Choshi, Japan); araT was from Sigma Chemical Co. (St. Louis, MO); acyclovir was from GlaxoSmithKline (Uxbridge, Middlesex, UK); ganciclovir was from Roche (Brussels, Belgium); penciclovir was obtained from Sanofi-Aventis (Paris, France). The BCNAs C1743 and C1368 were synthesized and provided by C. McGuigan (Cardiff University, Wales, UK).

Thymidine Kinase Assays. Kinase kinetics assays measuring the conversion of radiolabeled thymidine to thymidine monophosphate were carried out in a final volume of 50 μl containing 50 mM Tris-HCl, pH 8.0, 2.5 mM MgCl2, 10 mM diethiothreitol, 5 mM ATP, 10 mM NaF, and 1.0 mg/ml bovine serum albumin. The enzyme and radiolabeled substrate concentrations were chosen to satisfy the Michaelis-Menten conditions for initial velocities. The reaction was started by the addition of enzyme to the assay mixture at 37°C and was terminated by boiling. Aliquots of 45 μl were then spotted onto DE-81 discs (Whatman, Maidstone, UK) at different times. Once dried, the discs were washed three times for 5 min each in 1 mM HCOONa, while shaking, followed by 5 min in ethanol (70%). Finally, the filters were dried, placed in a scintillant, and counted for 3H in a liquid scintillation counter (LS6500; Beckman Coulter, Fullerton, CA).

The inhibition of phosphorylation of dThd by the wild-type and mutated VZVTKs was assessed by determining the IC50 value of the test compounds. The following reaction conditions were used: the standard reaction mixture (50 μl) contained 50 mM Tris-HCl, pH 8.0, 2.5 mM MgCl2, 10 mM diethiothreitol, 2.5 mM ATP, 10 mM NaF, 1.0 mg/ml bovine serum albumin, 1 μM [CH3-3H]dThd (0.1 μCi), and an appropriate amount of test compound. The reaction was started by the addition of enzyme. The incubation was carried out at 37°C for 30 min; the rest of the reaction follows the kinase assay described above. The IC50 value was defined as the drug concentration required to inhibit thymidine phosphorylation by 50%.

Results

The purified wild-type and mutant VZVTKs were compared regarding their ability to phosphorylate dThd. To examine the effects of the mutations on ligand binding and specificity, the Km value for each mutant against dThd and the IC50 value for a wide range of inhibitors was determined: the pyrimidine nucleosides BVDU, IVDU, BVaraU, araT, FIAU; the purine nucleosides acyclovir, ganciclovir, and penciclovir; and the BCNAs C1743 and C1368 (Fig. 1).

Thymidine Kinase Activity. The thymidine Km value for wild-type VZVTK used as a reference was 1.96 μM (Table 1) compared with literature Km values of between 0.3 and 0.6 μM (Suzutani et al., 1993; Amrhein et al., 2000). Whereas our Km value was greater than the average, VZVTK wild-type and mutants were purified, and their activity was as-

<table>
<thead>
<tr>
<th>VZVTK Mutant</th>
<th>Km (μM)</th>
<th>Vmax (nmol/mg protein/h)</th>
<th>km/Km (min⁻¹ μM⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>1.96 ± 0.83</td>
<td>14.8 ± 2.0</td>
<td>17.11</td>
</tr>
<tr>
<td>E59S</td>
<td>5.28 ± 0.93</td>
<td>21.6 ± 1.0</td>
<td>9.27</td>
</tr>
<tr>
<td>R84V</td>
<td>9.14 ± 1.49</td>
<td>32.1 ± 7.1</td>
<td>7.96</td>
</tr>
<tr>
<td>H97A</td>
<td>3.44 ± 1.75</td>
<td>23.8 ± 0.2</td>
<td>15.68</td>
</tr>
<tr>
<td>H97Y</td>
<td>6.30 ± 0.63</td>
<td>43.3 ± 1.6</td>
<td>15.54</td>
</tr>
<tr>
<td>Y21H</td>
<td>7.67 ± 0.49</td>
<td>18.4 ± 0.3</td>
<td>5.43</td>
</tr>
<tr>
<td>Y21E</td>
<td>5.36 ± 4.05</td>
<td>0.11 ± 0.01</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Fig. 2. Active sites superimposition of VZVTK (blue) and HSV1TK (gray) highlighting mutated residues.
sayed using exactly the same protocol to allow comparison of the results.

All six mutants had an increased $K_m$ value for dThd (Table 1), with the R84V thymidine kinase mutant showing the weakest apparent affinity (9.1 ± 1.5 μM). Nevertheless, the $K_m$ value did not vary by more than 5-fold, indicating that the mutations did not dramatically change the binding mode for dThd. Concerning the $V_{\text{max}}$, the reference value for the wild-type is 14.8 ± 2.0 nmol/μg protein/h. Most of the thymidine kinase mutants apart from the Y21E thymidine kinase mutant gave a higher $V_{\text{max}}$ value, ranging from a 3-fold increase for H97Y thymidine kinase to nearly complete loss of catalytic activity for Y21E thymidine kinase, with a $V_{\text{max}}$ value of only 0.11 ± 0.01 nmol/μg protein/h. The values of $k_{\text{cat}}/K_m$ did not change for H97A and H97Y thymidine kinase compared with the wild-type thymidine kinase (Table 1), showing that the reduction of the apparent affinity was compensated for by the increased catalytic rate. Mutations in the dimer interface of thymidine kinase (i.e., E59S and R84V) resulted in ~50% reduction of enzyme efficiency (1.8- and 2.1-fold decrease, respectively). For Y21H thymidine kinase, the $k_{\text{cat}}/K_m$ value was even lower (2.5-fold), whereas there was a much more significant decrease of 350-fold for Y21E thymidine kinase.

**Inhibitory Potency of Compounds against Mutant VZVTKs.** The purine nucleoside analog acyclovir (Cohen et al., 1999; Naessens and De Clercq, 2001) has been the main drug of choice against varicella zoster virus, even though its antiviral activity is much weaker than for herpes simplex viruses 1 and 2 (Andreil et al., 2000). In our studies, acyclovir, ganciclovir, and penciclovir (Fig. 1) showed an IC$_{50}$ value of greater than 500 μM for both wild-type and mutant VZVTK (Table 2). The reported $K_m$ value for acyclovir against VZVTK is 830 μM (Roberts et al., 1991), whereas ganciclovir has a similar affinity for VZVTK. Thus, none of the VZVTK mutants showed significantly different behavior in relation to this class of compound.

VZVTK has a preference for pyrimidine nucleoside analogs compared with purine nucleosides. For example, BVDU (De Clercq, 2004) has more than 10$^4$-fold superiority in potency over acyclovir (Roberts et al., 1991; Ashida et al., 1997; Kussmann-Gerber et al., 1998; Sienaert et al., 2002). In the case of the Y21E thymidine kinase mutant, the IC$_{50}$ value for this class of inhibitors seems to be very similar to the wild type, whereas for Y21H thymidine kinase, the IC$_{50}$ value was increased approximately 10-fold. Among other pyrimidine nucleoside analogs, araT and FIAU IC$_{50}$ values indicated either weaker or very similar inhibitory capacity compared with the other compounds. Mutations seemed to have little affect on the FIAU IC$_{50}$ value, which is, at worst, double that of wild type, whereas for araT, the IC$_{50}$ value varied from 2- to 8-fold.

Cf1368 and Cf1743 (Fig. 1) belong to the BCNA series of compounds, which have been shown previously to be efficiently phosphorylated by VZVTK but not by HSV1TK (Balzarini and McGuigan, 2002a,b). Thus, BCNAs represent a very potent and selective class of inhibitors of VZVTK. The Y21E thymidine kinase mutant seemed to be 5-fold more sensitive to BCNA Cf1368 than the wild type (Table 2). In contrast, for the Y21H mutant thymidine kinase, weaker inhibition compared with wild-type for all compounds tested was observed. The BCNAs seem particularly sensitive to the mutation E59S besides Y21H. Indeed, the IC$_{50}$ value increased 5- and 30-fold, respectively, for Cf1368 and Cf1743, the highest such values observed for the E59S mutation in VZVTK.

**Discussion**

**Effect of Mutating Residues at the VZVTK Dimer Interface.** In the VZVTK subunit interface (Fig. 3), there was a change in conformation within the loop defined by residues 55 to 61 relative to HSV1TK, which makes dimer interface contacts have differences between 1.0 and 4.4 Å in α-carbon positions. The position of active-site residues Tyr66 and Gln95 after the loop are related to it. Therefore, the residues Glu59 and Arg84 were mutated to their equivalents in HSV1TK, respectively, serine and valine, aimed at destabilizing the VZVTK loop conformation. Such mutations lost some apparent affinity for thymidine but showed a higher $V_{\text{max}}$ value compared with the wild-type thymidine kinase, whereas the overall enzyme catalytic efficiency ($k_{\text{cat}}/K_m$) is halved. These mutations showed that the interactions at the dimer interface are important for the binding of thymidine but not for the catalysis rate, which was increased. Conformational changes affecting catalytic function or inhibitor binding by mutations distal to the active site have been reported in other systems, such as glucokinase and reverse transcriptase (Ren et al., 1998; Pedelini et al., 2005). These observations may be explained by the fact that the VZVTK active site residues (Tyr66 and Gln95) after the loop could have more flexible positions, destabilizing the interactions with thymidine. Consequently, the apparent affinity decreased, but the catalytic rate increased, perhaps because the

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Wild Type</th>
<th>E59S</th>
<th>E84V</th>
<th>H97A</th>
<th>H97Y</th>
<th>Y21H</th>
<th>Y21E</th>
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<tr>
<td>BVDU</td>
<td>1.5 ± 0.1</td>
<td>5.9 ± 0.6</td>
<td>7.0 ± 0.1</td>
<td>4.7 ± 0.3</td>
<td>10 ± 5</td>
<td>14 ± 0</td>
<td>2.2 ± 0.9</td>
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<tr>
<td>IFDU</td>
<td>1.2 ± 0.1</td>
<td>5.6 ± 0.0</td>
<td>5.0 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>8.8 ± 0.1</td>
<td>1.8 ± 0.2</td>
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<tr>
<td>BvavaU</td>
<td>1.4 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td>6.6 ± 1.9</td>
<td>3.0 ± 1.3</td>
<td>19 ± 8</td>
<td>22 ± 1</td>
<td>1.7 ± 0.2</td>
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<tr>
<td>araT</td>
<td>20 ± 1</td>
<td>39 ± 1</td>
<td>116 ± 55</td>
<td>80 ± 10</td>
<td>11 ± 18</td>
<td>173 ± 38</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>FIAU</td>
<td>17 ± 1</td>
<td>35 ± 1</td>
<td>20 ± 11</td>
<td>34 ± 8</td>
<td>31 ± 3</td>
<td>34 ± 0</td>
<td>2.3 ± 0.1</td>
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<tr>
<td>Acyclovir</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
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</tr>
<tr>
<td>Ganciclovir</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
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<tr>
<td>Penciclovir</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
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<tr>
<td>CF1743</td>
<td>5.5 ± 3.3</td>
<td>298 ± 81</td>
<td>11 ± 7</td>
<td>27 ± 4</td>
<td>4.5 ± 0.5</td>
<td>223 ± 222</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>CF1368</td>
<td>33 ± 1</td>
<td>161 ± 131</td>
<td>19 ± 7</td>
<td>28 ± 4</td>
<td>38 ± 1</td>
<td>&gt;500</td>
<td>7.5 ± 5.0</td>
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</table>
greater flexibility allowed easier access of the substrate to the active site.

In terms of inhibition, the IC_{50} value was generally increased by 2- to 6-fold. It is clear that the BCNAs (Fig. 1) behave differently compared with the other class of compounds. For example, the E59S mutation dramatically decreases the inhibitory capacity of Cf1743, whereas the R84V mutation increased that of Cf1368. Arg84 and Glu59 from different subunits were linked by hydrogen bonds across the dimer interface; moreover, Glu59 also helped to stabilize its monomer by the formation of an intrasubunit hydrogen bond to His88. Therefore, we would expect a greater destabilization of the loop triggered by E59S than by Arg84, which was confirmed in the case of BCNAs. In conclusion, E59S and R84V are interesting mutations in the case of BCNAs: the loop stabilization by Glu59 and His88 seemed essential to BCNAs, whereas the stabilization by Arg84 decreased the k_{cat}/K_{m} value against Cf1368.

Adjusting the Size of the Pocket for Bromovinyl Substituents. The crystal structure of VZVTK (pdb code: 1OSN) shows that the 5-bromovinyl substituent of BVDU makes van der Waals contacts in the subpocket with His97. In a comparison of the HSV1TK and VZVTK active sites (Fig. 2), His97 was closer to the bromine than its HSV1TK equivalent Tyr132. Purine nucleoside analogs are used against both varicella zoster virus and herpes simplex virus 1, but they are more potent against herpes simplex virus 1. The design of the thymidine kinase mutants H97Y and H97A was aimed to adjust the size of the subpocket around the bromine. This pocket appeared smaller in the VZVTK structure compared with HSV1TK, and one reason was the substitution of a tyrosine (HSV1TK) to a histidine (VZVTK). We thus decided to mutate H97Y, and we also made the H97A mutation in VZVTK with the opposite aim of maximizing the size of the subpocket. The k_{cat}/K_{m} is the same as the wild type for both H97Y and H97A thymidine kinase mutants: they both have a reduced apparent affinity and a higher V_{max} value compared with the wild-type. Even if His97 does not participate in the catalysis, its mutation to a tyrosine increased the V_{max} value by 3-fold. It seems that His97 played an indirect role in the catalysis, perhaps in the correct orientation of the substrate to allow maximum rate enhancement.

Unfortunately, neither of the two codon 97 mutations improved the apparent affinity for bromovinyl substituent-containing compounds. For H97A thymidine kinase, the IC_{50} value has increased from 2- to 4-fold compared with the wild-type and from 5- to 12-fold for H97Y. The mutation of residue 97 to the HSV1TK structurally homologous amino acid was worse than wild type or H97A VZVTK. For both Cf1368 and Cf1743, the same IC_{50} value was seen for the H97Y mutant compared with the wild-type thymidine kinase, whereas the k_{cat} value was increased by 6-fold for BVDU for example (Table 2). With the H97A mutant, the IC_{50} value for Cf1368 did not significantly change relative to the wild type, but there was a 5-fold increase for Cf1743. In conclusion, adjusting the size of the subpocket did not improve the binding of bromovinyl substituents as expected; in fact, it had the opposite effect. We were surprised to find, however, that the only class of compounds not affected were BCNAs, whereas the IC_{50} values for all the other compounds were increased. There is one exception: for H97A VZVTK against Cf1743, the only difference between Cf1743 and Cf1368 was the presence of a p-alkyl-substituted phenyl group as the side chain of the furanyl ring instead of an alkyl side chain (Fig. 1). We can infer that the Cf1743 phenyl group could be stabilized by aromatic ring-stacking interactions with the Tyr97 ring, and the side-chain substitution did not significantly affect Cf1368, because no interaction could be made with its aliphatic chain.

Alteration of Contacts with the Sugar Ring. Residue Tyr21 was selected for mutagenesis because its equivalent in HSV1TK, His58 (Fig. 2), has a completely different hydrogen bonding pattern in the active site. Tyr21 made a unique hydrogen bond to the Ala193 carbonyl (3 Å), whereas His58 bonded with Tyr101 (VZVTK Tyr66) and with Tyr172 (VZVTK Phe139), which is important for the ribose 3'-hydroxyl and pyrimidine binding, respectively. We mutated Tyr21 to a histidine, the corresponding residue in HSV1TK, and mutated Tyr21 to a glutamic acid, which is more flexible than a tyrosine yet still able to hydrogen bond to Tyr66 and/or to the ribose 3'-hydroxyl. The k_{cat}/K_{m} value was diminished for both mutants (Table 1); in the case of Y21H thymidine kinase, it was decreased by almost a factor of 3, whereas Y21E thymidine kinase was 350-fold less efficient than the wild type. This dramatic change in the catalytic efficiency was due mainly to the 100-fold decrease in the V_{max} value, whereas Y21H thymidine kinase showed a higher V_{max} value than the wild-type TK. It seems that the presence of a ring is important for the rate of catalysis of dThd phosphorylation, but it only has small effects on the apparent binding affinity.

We were surprised to find that the IC_{50} values for all compounds against Y21E thymidine kinase were very similar to those of the wild type, except for BCNAs Cf1743 and Cf1368, which showed a 2.5-fold higher (Cf1743) or 5-fold lower (Cf1368) IC_{50} value (Table 2). The Y21H mutation significantly decreases the affinity for BCNAs. BCNAs are known to be very selective for VZVTK and are not at all able to bind HSV1TK (Balzarini et al., 2002). The exact mechanism for their antiviral activity remains unclear but is known to be highly dependent on phosphorylation by VZVTK (Bal-
zarini and McGuigan, 2002a). The mutation of Y21H, which was made to mimic the HSV1TK active site, seems catastrophic for BCNAs. Because HSV1TK was unable to bind BCNAs, it could be possible that a contributory factor for this weak affinity was the presence of a histidine in the structurally equivalent position (His58). Bird et al. (2003) suggested that BCNAs might adopt a significantly different binding mode in VZVTK compared with conventional nucleosides because they could not be easily modeled into the VZVTK active site based on overlaps with the BVDU deoxyribose or base positions. The Y21H mutation may force the BCNA's sugar position into a certain conformation, which is not adequate for the reaction to occur.

In conclusion, this work has provided interesting clues as to substrate specificity for TKs, especially relating to BCNAs. Our study highlights a few points: the stabilization of the dimer interface was not essential for Cf1368 binding, but for both BCNA molecules studied, the integrity of the loop was important. Second, the BCNAs with a p-alkyl-substituted phenyl group apparently require aromatic ring stacking interactions at residue 97 for optimal inhibitory effect. Finally, a charged residue at position 21 with a long and flexible side chain improved the binding of BCNA Cf1368.

Acknowledgments

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References


Address correspondence to: Dr. David K. Stammers, Division of Structural Biology, The Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK. E-mail: daves@strubi.ox.ac.uk