Flavopiridol Inhibits Glycogen Phosphorylase by Binding at the Inhibitor Site

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Flavopiridol (L86–8275) ((--)-cis,5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-4H-benzopyran-4-one), a potential antitumor drug, currently in phase II trials, has been shown to be an inhibitor of muscle glycogen phosphorylase (GP) and to cause glycogen accumulation in A549 non-small cell lung carcinoma cells (Kaiser, A., Nishi, K., Gorin, F.A., Walsh, D.A., Bradbury, E. M., and Schnier, J. B., unpublished data). Kinetic experiments reported here show that flavopiridol inhibits GPb with an IC<sub>50</sub> = 15.5 μM. The inhibition is synergistic with glucose resulting in a reduction of IC<sub>50</sub> for flavopiridol to 2.3 μM and mimics the inhibition of caffeine. In order to elucidate the structural basis of inhibition, we determined the structures of GPb complexed with flavopiridol, GPb complexed with caffeine, and GPb complexed with both glucose and flavopiridol at 1.76-, 2.30-, and 2.23-Å resolution, and refined to crystallographic R values of 0.216 (R<sub>free</sub> = 0.247), 0.189 (R<sub>free</sub> = 0.219), and 0.195 (R<sub>free</sub> = 0.252), respectively. The structures provide a rationale for flavopiridol potency and synergism with glucose inhibitory action. Flavopiridol binds at the allosteric inhibitor site, situated at the entrance to the catalytic site, the site where caffeine binds. Flavopiridol intercalates between the two aromatic rings of Phe<sup>282</sup> and Tyr<sup>415</sup>. Both flavopiridol and glucose promote the less active T-state through localization of the closed position of the 280s loop which blocks access to the catalytic site, thereby explaining their synergistic inhibition. The mode of interactions of flavopiridol with GP is different from that of des-chloro-flavopiridol with CDK2, illustrating how different functional parts of the inhibitor can be used to provide specific and potent binding to two different enzymes.

Flavopiridol (L86–8275, Scheme I), a flavonoid, has been shown to be a potent, competitive inhibitor (with respect to ATP) of cyclin-dependent kinases (CDKs)<sup>1</sup> with IC<sub>50</sub> values between 0.2 and 0.4 μM (1) and to have antiproliferative and cytotoxic activity on certain tumor cell lines in vitro and in vivo (2–5). The compound is currently in phase II trials, the first CDK inhibitor to be tested in clinical trials (5, 6). X-ray crystallographic analysis has provided evidence that des-chloroflavopiridol (L86–8276) binds to the ATP-binding site of CDK2 (7, 8), and also a structural basis for the development of novel CDK modulators, as therapeutic agents for cancer therapy (9–11).

Recently, it was found that flavopiridol significantly inhibited both rabbit muscle glycogen phosphorylase b (GPb) (IC<sub>50</sub> = 1 μM) and glycogen phosphorylase a (GPa) (IC<sub>50</sub> = 2.5 μM), but AMP activated GPb was poorly inhibited by flavopiridol.<sup>2</sup> Furthermore, flavopiridol treatment of A549 non-small cell lung carcinoma cells resulted in an increase in glycogen accumulation. These findings raise the possibility that this antitumor drug may also interfere with glucose homeostasis in addition to the effects on the cell cycle through CDK inhibition.

GP (EC 2.4.1.1), a key enzyme in the regulation of glycogen metabolism, catalyzes the degradative phosphorylisis of glycogen to glucose 1-phosphate (glucose-1-P). In muscle, glucose-1-P is utilized via glycolysis to generate metabolic energy, but in the liver it is mostly converted to glucose, which is the output for the benefit of other tissues (12). The enzyme exists in two interconvertible forms: the dephosphorylated form, GPb (low activity, low substrate affinity), and the Ser<sup>14</sup>-phosphorylated form, GPa (high activity, high substrate affinity). AllostERIC activators, such as AMP or inhibitors such as ATP, glucose-6-P, and caffeine can alter the equilibrium between a less active T state and a more active R state or vice versa. The structures of T and R state GP have been characterized (13–15).

Because of its central role in glycogen metabolism, GP has been exploited as a target for structure-assisted design of compounds that might prevent unwanted glycolysis under high glucose conditions that may be relevant to the control of diabetes (16–24). GP contains at least 6 potential regulatory

<sup>1</sup>The abbreviations used are: CDK, cyclin-dependent kinase; GP, glycogen phosphorylase, 1,4-a-D-glucan:orthophosphate α-glucosyltransferase (EC 2.4.1.1); GPb, glycogen phosphorylase b; GPa, glycogen phosphorylase a; glucose-1-P, α-D-glucose 1-phosphate; flavopiridol, L86–8275, (--)-cis,5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl-piperidinyl)-4H-benzopyran-4-one]; CDK2, cyclin-dependent kinase 2; 2-fluorobenzyl]-4-(4-hydroxyperipherin-1-yl)-2-oxoethyl]amide; Bes, N,N-bis(2-hydroxyethyl)-2-aminothanesulfonic acid.

<sup>2</sup>A. Kaiser, K. Nishi, F. A. Gorin, D. A. Walsh, E. M. Bradbury, and J. B. Schnier, submitted for publication.

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The atomic coordinates and structure factors (codes 1C8K, 1GFZ, and 1E1Y) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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sites: the Ser14-phosphate recognition site, the allosteric site that binds AMP, IMP, ATP, and glucose-6-P, the catalytic site that binds the substrates glycogen and glucose-1-P, and also glucose and glucose analogues, the inhibitor site, which binds caffeine and related compounds, the glycogen storage site, and a new allosteric inhibitor site situated at the dimer interface which binds the potential antidiabetic drug CP320626 (24) (Fig. 1).

In order to provide a stereochemical explanation for flavopiridol inhibition of glycogen phosphorylase, we have co-crystallized GPb with flavopiridol, and GPa with glucose and flavopiridol and determined the structures of the complexes by x-ray crystallographic methods at 1.76- and 2.23-Å resolution, respectively. The structural results show that flavopiridol binds at the inhibitor site, the site where caffeine binds, located 10 Å from the catalytic site. In order to compare the flavopiridol-binding site with the caffeine-binding site we determined the structure of GPb complexed with caffeine at 2.30-Å resolution. The detailed interactions of flavopiridol with the protein provide a structural explanation for understanding the molecular basis of its high affinity for GP and show that flavopiridol is an inhibitor that stabilizes the T state conformation as do caffeine and glucose. We also demonstrate through kinetic studies a strong synergistic inhibition of GPb by the pair flavopiridol/glucose.

**Experimental Procedures**

**Kinetic Experiments**

GPb was isolated from rabbit skeletal muscle according to Fischer and Krebs (25) using 2-mercaptoethanol instead of l-cysteine and recrystallized as described (23). Protein concentration was determined from absorbance measurements at 280 nm using an absorbance index $A_{1%}^{1cm} = 13.2$ (26). Glucose-1-P (dipotassium salt), AMP, (oyster) glycogen, and other chemicals were obtained from Sigma. Glycogen was freed of AMP by the method of Helmreich and Cori (27). Phosphorylase activity in the direction of glycogen synthesis was measured at pH 6.8 and 30 °C with 5 mg/ml enzyme, 10 mM glucose-1-P, 1% glycogen, 1 mM AMP, and a range of concentrations of inhibitor(s) as indicated, in 50 mM triethanolamine hydrochloride/HCl, 100 mM KCl, 1 mM EDTA, and 1 mM DTT buffer. The enzyme was preincubated with glycogen for 15 min at 30 °C before the reaction was started by adding glucose-1-P. Inorganic phosphate released in the reaction was measured and initial velocities were calculated from the pseudo-first order reaction constants as previously (20).

**Crystallographic Experiments**

**Crystallization and Data Collection**—Native T state tetragonal (P4_2_2) GPb crystals were grown as described previously (28) with 1 mM IMP. GPb-flavopiridol and GPa-glucose-flavopiridol complexes were co-crystallized as previously (21, 23) in a medium consisting of 25 mg/ml enzyme, 1 mM flavopiridol, 3 mM DTT, 10 mM Bes, 0.1 mM EDTA, 0.02% sodium azide, pH 6.7, and 1 mM spermine (GPb) or 10 mM magnesium acetate and 50 mM glucose (GPa); as flavopiridol is insoluble under...
these conditions, the mixtures were filtered out to remove precipitates. The GPb-flavopiridol crystals were transferred to a fresh buffer solution (3 mM DTT, 10 mM Bes, 0.1 mM EDTA, 0.5 mM flavopiridol, pH 6.7) just before room temperature data collection. The GPb-glucose-flavopiridol crystals were transferred to a fresh buffer solution (3 mM DTT, 10 mM Bes, 0.1 mM EDTA, 10 mM magnesium acetate, 50 mM glucose, 0.3 mM flavopiridol, 0.02% sodium azide, pH 6.7) containing 30% (v/v) glycerol for 15–30 min to promote in a loop, and flash frozen with nitrogen gas at 100 K. Data for GPb-flavopiridol complex were collected from a single crystal, on an image plate on the beamline X31 at Hamburg (λ = 1.05 Å), at a maximum resolution of 1.7 Å by followed data collection at a lower resolution (3.0 Å) to measure the strong low angle reflections. Data for GPb-glucose-flavopiridol complex were collected from a single crystal at a resolution of 2.23 Å on the same beamline station. Data for GPb-caffeine-IMP complex were collected at a resolution of 2.30 Å from a single native GPb crystal soaked in a solution containing 5 mM caffeine, 10 mM Bes, 0.1 mM EDTA, pH 6.7, for 80 min, on an Image Plate RAXIS IV mounted on a Rigaku Ru-H3RHB generator with a belt drive rotating anode (λ = 1.5418 Å), at the National Center for Scientific Research “Demokritos” at Athens. Crystal orientation and integration of reflections were performed using DENZO (29). Inter-frame scaling, partial reflection summation, data reduction, and post-refinement were all completed using SCALEPACK (29).

Refinement—Crystallographic refinement of the GPb-flavopiridol complex was performed with X-PLOR version 3.8 (30) using bulk solvent corrections. All data between 29.5 and 1.76 Å were included with frame scaling, partial reflection summation, data reduction, and post-integration of reflections were performed using DENZO (29). Interframe scaling, partial reflection summation, data reduction, and post-refinement were all completed using SCALEPACK (29).

Results

Flavopiridol Binding to Glycogen Phosphorylase

Flavopiridol and glucose are able to bind to the enzyme at the same time, and activation loci were covalently attached in the reaction. The kinetic data indicate that flavopiridol inhibition of GPb is synergistic with glucose, suggesting that both flavopiridol and glucose are able to bind to the enzyme at the same time (39). The effect of varying glucose concentration on the IC_{50} value for flavopiridol is shown in Fig. 2B. At 10 mM glucose concentration, the relative IC_{50} value for flavopiridol was decreased by more than 5-fold. Caffeine, a T state inhibitor of the enzyme, was used as a control and did not decrease the relative IC_{50} value for flavopiridol. The IC_{50} values were as in A. The normalized values (obtained by dividing these values by the IC_{50} value obtained in the absence of glucose) are plotted as a function of glucose concentration. B, the effect of glucose on the potency of flavopiridol. IC_{50} values for GPb inhibition were determined in the direction of glycocon synthesis as described under "Experimental Procedures" with 5 μg/ml enzyme at constant concentrations of glucose-1-P (10 mM) and glycerophosphorylase (1%), and varied flavopiridol concentrations (2–40 μM) in the absence or presence of various concentrations of glucose. The IC_{50} values were as in A. The normalized values (obtained by dividing these values by the IC_{50} value obtained in the absence of glucose) are plotted as a function of glucose concentration.

Synergistic Inhibition by Flavopiridol and Glucose—Kinetic experiments with GPb showed that flavopiridol is an inhibitor of the enzyme with an IC_{50} = 15.5 ± 0.3 μM, measured in the direction of glycogen synthesis, and in the presence of saturating concentrations of glucose-1-P (10 mM), glycogen (1%) and AMP (1 mM), at 30 °C (Fig. 2A). The IC_{50} value for glucose was calculated to be 19.7 ± 1.3 mM. Kaiser et al. found an IC_{50} = 1 μM for flavopiridol inhibition of rabbit muscle GPb, when tested in the direction of glycogen breakdown, in the presence of 0.8 mM AMP, 20 mM phosphate, pH 7.2, 0.1% glycerol, and 25 °C. This difference may well be a consequence of the reaction being measured in the opposite direction of catalysis and the different substrate concentrations used. To investigate the interaction between flavopiridol and glucose binding to GPb, initial velocity studies were carried out by varying flavopiridol and glucoses concentrations at fixed concentrations of the substrates glucose-1-P (10 mM) and glycogen (1%). The IC_{50} values for flavopiridol inhibition of the enzyme were 10.9, 6.5, 4.3, and 2.3 μM, in the presence of 2, 4, 6, and 10 mM glucose, respectively. The kinetic data indicate that flavopiridol inhibition of GPb is synergistic with glucose, suggesting that both flavopiridol and glucose are able to bind to the enzyme at the same time (39). The effect of varying glucose concentration on the IC_{50} value for flavopiridol is shown in Fig. 2B. At 10 mM glucose concentration, the relative IC_{50} value for flavopiridol was decreased by more than 5-fold. Caffeine, a T state inhibitor of the enzyme, was used as a control and did not decrease the relative IC_{50} value for flavopiridol.
enzyme (with a $K_v$ value of 0.1–0.2 mM) is also known to function with glucose in a synergistic mode, with each compound promoting the binding of the other (with an interaction constant $\alpha$ = 0.2) (40, 41).

**Flavopiridol-binding Site**—The overall architecture of the native T state GPb with the location of the cofactor pyridoxal 5'-phosphate, the catalytic site, the allostERIC (or nucleotide) site, the inhibitor (or nucleoside) site, and a new allosteric inhibitor site, identified recently as target for drug interactions, is presented in Fig. 1. The inhibitor site is located at the entrance to the catalytic site, near the domain interface, and comprises residues from both domains 1 (residues 13–484) and 2 (residues 485–842). The site binds a number of aromatic molecules and 107 van der Waals interactions (58 nonpolar/nonpolar, 22 polar/polar, and 6 polar/polar) (Figs. 4A and 6A).

Crystallographic data collection, processing, and refinement statistics for the co-crystallized GPb-flavopiridol and GPa-glucose-flavopiridol complexes are shown in Table I. For both complexes, the refined $2F_o - F_c$ electron density maps indicated that flavopiridol (Scheme I) bound strongly at the inhibitor site (Fig. 3), consistent with the kinetic results. In the GPa-glucose-flavopiridol complex, additional density at the catalytic site indicated binding of glucose. The mode of binding and the interactions that glucose makes with GPb, in the presence of flavopiridol, are almost identical with those previously reported for the complex GPa-glucose at 100 K (23).

In particular, Asp$^{283}$ and Asn$^{284}$ of the 280s loop are in the same position where they are found in the GPa-glucose complex. No other binding sites were observed for flavopiridol.

**Interactions of Flavopiridol with Inhibitor Site Residues**—The most characteristic feature for flavopiridol binding to GP is its stacking interactions with the two aromatic residues, Phe$^{285}$ from the 280s loop (residues 282 to 286) and Tyr$^{613}$. Flavopiridol binding on the inhibitor site of GPb makes a few polarpolar interactions and exploits numerous van der Waals contacts; it makes a total of 4 hydrogen bonds to water molecules and 107 van der Waals interactions (58 nonpolar/nonpolar, 6 polar/polar, and 43 polar/nonpolar) (Figs. 4A and 6A).

The benzopyran-4-one ring exploits 61 van der Waals contacts which are dominated by the substantial contacts made to almost all atoms of Phe$^{285}$ and Tyr$^{613}$. In GPb-flavopiridol, the plane of benzopyran-4-one ring lies almost parallel to the plane of the Phe$^{285}$ ring, at a distance of 3.4 Å and makes an angle of about 10° with the Tyr$^{613}$ ring, at distances varied between 3.7 Å (C4 to CG) and 3.9 Å (C2 to CZ). Carbonyl oxygen O$^4$ makes an indirect contact to the main chain atoms of Asp$^{283}$ via a water molecule (Wat$^{36}$) and to Ile$^{570}$ (O) and Ala$^{610}$ (N) via another water molecule (Wat$^{283}$). A water-mediated (Wat$^{210}$) link between hydroxyl O-5 with Asn$^{282}$ (OD1) and main chain N of Gly$^{612}$ is also apparent.

The chlorophenyl ring is positioned in an adjacent pocket.
between, His^571 and Glu^572, Arg^770 and Phe^771, Ile^380 and Glu^382, Tyr^613, and Asn^284 of the 280s loop. This part of the molecule exploits 29 van der Waals contacts which are dominated by the substantial contacts made to almost all atoms of Glu^382. Indeed, the side chain of Glu^382 stacks against the chlorophenyl group making some 12 van der Waals contacts. CL1 is positioned between the Tyr^613 hydroxyl group and the OE-2 of Glu^572 at nearly the same distance of 3.3 Å but these contacts are not of the type “carbon-bound halogen: electron-dative atoms” identified in Ref. 42.

The 4-hydroxypiperidin-1-y1 group projects into solvent and makes 2 rather long (3.9 Å) non-polar/non-polar contacts to side chain atoms of Phe^265 and 8 contacts to water molecules. In addition, Wat^307 contacts both N-1 (2.7 Å) and hydroxyl O-3 (3.3 Å). This water molecule was not previously seen in the native structure. On forming the complex with GPb the inhibitor becomes buried. The solvent accessibilities of the free and bound flavopiridol molecules are 560 Å^2 and 191 Å^2, indicating that a significant portion of this inhibitor is buried. The inhibitor becomes inaccessible on binding flavopiridol, of which 184 Å^2 becomes inaccessible to water or that flavopiridol molecules are 560 Å^2 and 191 Å^2, indicating that a significant portion of this inhibitor is buried.

In order to compare the flavopiridol-binding site with the caffeine-binding site in GPb we have determined the room temperature GPb-caffeine-IMP complex structure at a 2.3-Å resolution (Table I). IMP, which was included in the crystallization mixture at a concentration of 1 mM, is bound at the allosteric site and the mode of binding and the interactions that IMP makes with GPb are similar to those observed for AMP in the T state GPb-AMP complex (43). Caffeine, like flavopiridol, is intercalated between the two aromatic residues, Phe^280 and Tyr^613, and on binding to GPb it makes a total of 79 van der Waals contacts (44) van der Waals contacts, including 12 van der Waals contacts made to aromatic residues and 29 van der Waals contacts to the chlorophenyl ring, and 12 NH-H hydrogen bonds to OD-1 of Asn 133 and NE-2 of His 571 (45). Glucose, bound at the catalytic site, interacts with the side chains of Asp^282 and Glu^382, holding the loop in its inactive state. Glucose, bound at the catalytic site, interacts with the side chains of Asp^282 and Glu^382, holding the loop in its inactive state.

Comparisons with GPb-Caffeine Complex in the T State—In order to compare the flavopiridol-binding site with the caffeine-binding site in GPb we have determined the room temperature GPb-caffeine-IMP complex structure at a 2.3-Å resolution (Table I). IMP, which was included in the crystallization mixture at a concentration of 1 mM, is bound at the allosteric site and the mode of binding and the interactions that IMP makes with GPb are similar to those observed for AMP in the T state GPb-AMP complex (43). Caffeine, like flavopiridol, is intercalated between the two aromatic residues, Phe^280 and Tyr^613, and on binding to GPb it makes a total of 79 van der Waals contacts (44) van der Waals contacts, including 12 van der Waals contacts made to aromatic residues and 29 van der Waals contacts to the chlorophenyl ring, and 12 NH-H hydrogen bonds to OD-1 of Asn 133 and NE-2 of His 571 (45). Glucose, bound at the catalytic site, interacts with the side chains of Asp^282 and Glu^382, holding the loop in its inactive state. Glucose, bound at the catalytic site, interacts with the side chains of Asp^282 and Glu^382, holding the loop in its inactive state.

DISCUSSION

Flavopiridol binds at the inhibitor site of GP; the inhibitor site is a hydrophobic binding pocket of relatively low specificity. Binding at this site shows great diversity: purines such as adenosine and caffeine, nucleosides such as adenosine and inosine, nucleotides such as AMP, IMP, and ATP, NADH and certain related heterocyclic compounds such as FMN (flavin mononucleotide) have been shown to bind at this site (40, 44, 45) in muscle GPb and GPa, but liver GPa shows a more stringent selectivity for inhibitors (40). The physiological significance of this inhibition has yet to be established but it may be used by an unidentified compound to enhance the effects of the control of liver GPa by glucose, possibly in response to insulin (46, 47).

The kinetic experiments on the separate and combined effects of flavopiridol and glucose (Fig. 2) showed that flavopiridol and glucose exhibit synergy in inhibiting GPb. Our experimental complex structures confirm these observations and show how both ligands, glucose and flavopiridol, stabilize the less active T state through interactions with the 280s loop (Fig. 5). Glucose, bound at the catalytic site, interacts with the side chains of Asp^282 and Asn^284 holding the loop in its inactive state. Flavopiridol, bound at the inhibitor site, some 12 Å from...
the catalytic site, interacts with Phe\textsuperscript{285} and augments the localization of the 280s loop in its inactive conformation. The key transition between inactive T state and active R state GP involves a movement and disordering of the 280s loop that allows a crucial arginine, Arg\textsuperscript{569}, to enter the catalytic site in place of Asp\textsuperscript{283}, and which also opens access for glycogen to the catalytic site. The shift and disordering of the 280s loop is associated with changes at the intersubunit contacts of the dimer that give rise to allosteric effects (14). By their dual action glucose and flavopiridol hold the 280s loop in the inactive conformation and block access to the catalytic site. The effect of glucose on the potency of flavopiridol could be an important physiological feature of a liver GP\textsubscript{a} inhibitor, as it has been suggested for the pair CP-91149/glucose (48), because the decrease in inhibitor potency as glucose concentrations decrease \textit{in vivo} should diminish the risk of hypoglycemia.

Flavopiridol has been shown to be a potent inhibitor of CDK2 kinase activity with an IC\textsubscript{50} value of 0.4 \mu M (1). To understand how flavopiridol binds to CDK2, De Azevedo \textit{et al.} (7) have determined the x-ray structure of CDK2 in complex with the des-chloroflavopiridol (L86-8276) at 2.33-Å resolution. The conformation of flavopiridol in the GP\textsubscript{b}-flavopiridol complex is similar with that observed for the refined structure of the CDK2-L868276 complex but not identical. In both the GP\textsubscript{b}-flavopiridol and CDK2-L868276 structures, the planes of benzopyran-4-one and the 3-hydroxy-1-methyl piperidinyl are al-
most perpendicular with the piperidinyl ring in chair geometry. In the CDK2-L868276 structure, the benzopyran-4-one is co-planar with the phenyl ring, but in the GPb-flavopiridol structure, the chlorophenyl group is inclined approximately 36° (torsion angle O1-C2-C21-C22 = 36°) to the benzopyran-4-one. L86–8276 binds in the ATP-binding pocket of CDK2, with the benzopyran-4-one ring occupying approximately the same region as the purine ring of ATP but inclined about 60° relative to the adenine. The piperidinyl moiety partially occupies the α-phosphate pocket, while the phenyl ring occupies a pocket not observed in the ATP complex structure. The binding of L86–8276 is characterized by predominantly hydrophobic and van der Waals interactions and specific hydrogen bonds to Lys33, Glu81 (main chain), Leu83 (main chain), and Asp145 and to two water molecules. In the CDK2-L868276 complex, the buried area of L86–8276 is 301 Å² and the buried surface in CDK2 is 399 Å², making a total decrease in solvent-accessible area of 700 Å² (7) compared with the total buried surface area in the GPb-flavopiridol complex of 618 Å². Furthermore, examination of the CDK2-L868276 structure suggests that the introduction of a chlorophenyl instead of a phenyl in the L86–8276 molecule would probably increase the total number of

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**FIG. 5.** Stabilization of the 280s loop in its inactive conformation by glucose, bound at the catalytic site, and flavopiridol, bound at the inhibitor site, of GPa. Coordinates of the refined GPa-glucose-flavopiridol complex structure are displayed.

**FIG. 6.** Stereodiagrams of the contacts between flavopiridol and GPb (A) and between des-chloroflavopiridol and CDK2 (B) after superimposing the protein structures with respect to the benzopyran-4-one rings of the two ligands.
contacts between flavopiridol and enzyme to 61. Comparison between the CDK2-L868276 and GPb-flavopiridol complexes show that flavopiridol makes different interactions with the two proteins (Fig. 6, A and B). In CDK2 it exploits specific hydrogen bonds that mimic those of ATP and non-polar interactions that are made mostly to aliphatic chains. In GPb-flavopiridol complex there are no specific hydrogen bonds apart to those to water molecules and the non-polar interactions involve π-π stacking with the aromatic groups of Phe285 and Tyr613.

A compound, such as flavopiridol, that targets both enzymes, CDK2 and GP, could have an added benefit in starving cancer cells of glycolytic intermediates at the same time disrupting the cell cycle and sending cells into apoptosis. If flavopiridol action is to act not only by inhibiting CDK2 but also by inhibiting GP, there needs to be comparable IC50 values for both enzymes. In the absence of glucose there is a 40-fold difference in IC50 values which could suggest that with doses needed to inhibit CDK2 there would be no action on GP but in the presence of 10 mM glucose the difference in IC50 values is only 6-fold and this could allow concentrations at which the inhibitor could be effective with both enzymes.

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