A Structural Basis for the Inhibition of the NS5 Dengue Virus mRNA 2'-O-Methyltransferase Domain by Ribavirin 5'-Triphosphate*

Delphine Benaroch‡§, Marie-Pierre Egloff‡, Laurence Mulard‡, Catherine Guerreiro‡, Jean-Louis Romette‡, and Bruno Canard‡

From the 3Centre National de la Recherche Scientifique and Universités d’Aix-Marseille I et II, UMR 6098, Architecture et Fonction des Macromolécules Biologiques, École Supérieure d’Ingénieurs de Luminy-Case 925, 163 avenue de Luminy, 13288 Marseille cedex 9, France and the Institut Pasteur, Unité de Chimie Organique, 25 rue du Dr. Roux, 75724 Paris cedex 15, France

Ribavirin is one of the few nucleoside analogues currently used in the clinic to treat RNA virus infections, but its mechanism of action remains poorly understood at the molecular level. Here, we show that ribavirin 5'-triphosphate inhibits the activity of the dengue virus 2'-O-methyltransferase NS5 domain (NS5MTaseDV). Along with several other guanosine 5'-triphosphate analogues such as acyclovir, 5-ethyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR), and a series of ribose-modified ribavirin analogues, ribavirin 5'-triphosphate competes with GTP to bind to NS5MTaseDV. A structural view of the binding of ribavirin 5'-triphosphate to this enzyme was obtained by determining the crystal structure of a ternary complex consisting of NS5MTaseDV, ribavirin 5'-triphosphate, and S-adenosyl-L-homocysteine at a resolution of 2.6 Å. These detailed atomic interactions provide the first structural insights into the inhibition of a viral enzyme by ribavirin 5'-triphosphate, as well as the basis for rational drug design of antiviral agents with improved specificity against the emerging flaviviruses.

The guanosine analogue ribavirin is a broad spectrum antiviral agent discovered almost 30 years ago (1). Since its discovery, many mechanisms of action have been proposed (reviewed in Refs. 2 and 3). Like most nucleoside analogues, ribavirin is phosphorylated by cellular kinases at its 5'-position upon entry into the cell. Ribavirin 5'-monophosphate is a potent inhibitor of the cellular enzyme inosine 5'-monophosphate dehydrogenase. This inhibition results in the depletion of the intracellular guanosine nucleotide pool, which feeds capping and polymerase enzymes from both viral and cellular origin. Consequently, the depressed guanosine nucleotide pool may exert an indirect antiviral effect, since viral enzymes would not compete advantageously for guanosine nucleotides with cellular enzymes. In addition, ribavirin nucleotides may have a viral target, such as RNA polymerization and RNA capping or induce lethal mutagenesis of viral genomes (4), accounting for the observed antiviral effect. Both direct and indirect mechanisms may thus contribute to the ribavirin mode of action. To date, ribavirin nucleotides have been crystallized with two cellular enzymes, namely inosine 5'-monophosphate dehydrogenase (5) and nucleoside diphosphate kinase (NDPK (6)) but not with any viral enzyme or protein.

The genus Flavivirus comprises important human pathogens such as West Nile, dengue, and yellow fever viruses, which are moderately sensitive to ribavirin (7–9). These mosquito-borne viruses are currently expanding their distribution throughout the world. The introduction of West Nile virus in North America may be an important milestone in the history of this virus, as exemplified by outbreaks in the New York area (10) followed by the gradual spread to 47 of the 49 continental states of the United States of America (www.cdc.gov/ncidod/dvbid/westnile/index.htm). The Camargue area in France has re-witnessed West Nile viral infection of horses after 40 years, and the first human cases were reported in the French Riviera in October 2003. Likewise, dengue virus, an agent responsible for hemorrhagic fever, infects more than 50 million persons annually with an increasing incidence in tropical areas around the world.

The single-stranded RNA genome of flaviviruses is of positive polarity, and is capped with a cap 1 structure $\text{Me}^7\text{GpppA}_2\text{OMe}$ (11). The N-terminal domain of the dengue virus polymerase NS5 is a 2'-O-methyltransferase that is active on RNA cap structures (12). This enzyme, referred to as NS5MTaseDV, is able to bind a GTP molecule that may mimic the RNA cap structure prior to methylation. Thus, it was of interest to determine whether guanosine analogues could bind to the GTP binding site of NS5MTaseDV. If so, guanosine analogues may act as potential competitive inhibitors of RNA cap binding and aid in the rational design of inhibitors directed against flaviviruses.

In this report, we present biochemical evidence that ribavirin 5'-triphosphate (RTP) inhibits the 2'-O-methyltransferase activity of dengue virus NS5MTaseDV. We also show that a series of RTP analogues, as well as EICAR 5'-triphosphate (EICAR-Tp) and acyclovir 5'-triphosphate (acyclovir-Tp), compete with GTP for binding to NS5MTaseDV. In addition to show that RTP and GTP share a common binding site on

---

*This investigation was supported in part by the French Ministry Program “Maladies Infectieuses” and a grant from the Direction Générales des Armées and in part by the European Economic Community program “Flavitherapeutics” (QLK3-CT-2001-00506). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a predoctoral fellowship from the Direction Générales des Armées and the Fondation pour la Recherche Medicale.

§ To whom correspondence should be addressed. Tel.: 33-491-82-86-44; Fax: 33-491-82-86-46; E-mail: bruno@afmb.cnrs-mrs.fr.

1 The abbreviations used are: RTP, ribavirin 5'-triphosphate; RMP, ribavirin 5'-monophosphate; SAHC, S-adenosyl-L-homocysteine; PDB, Protein Data Bank; EICAR, 5-ethyl-1-β-D-ribofuranosylimidazole-4-carboxamide; HPLC, high pressure liquid chromatography; GDPMP, $\beta$-$\gamma$-methylene GTP.
NS5MTase,DV, the crystal structure of the RTP-NS5MTase,DV complex reveals a unique mode of binding for the ribavirin pseudobase that is consistent with a lack of discrimination of this antiviral molecule relative to GTP.

EXPERIMENTAL PROCEDURES

Enzymes and Reagents—The purification and crystallization of the NS5 capping domain of the dengue virus RNA-dependent RNA polymerase has been described (12). Synthesis and purification of RTP, ribavirin nucleotide analogues, and acyclovir 5’-TP to homogeneity, as determined by HPLC, isopropyl and PVM NMR and HPLC, have been described (6). EICAR 5'-TP was a kind gift from P. Herdewijn (Leuven, Belgium). All other nonradioactive and 32P-labeled nucleotides were of HPLC grade and purchased from Amersham Biosciences.

Inhibition of RNA 2’-O-Methyltransferase Activity by RTP—The methyltransferase assay was performed in 40 mM Tris-HCl, pH 7.1, 100 μM S-adenosylmethionine with 10 μCi of Adenosyl-[3H]Met (74 Ci/mmol; Amersham Biosciences) were incubated with 2 μg of purified NS5MTase,DV, 35 μl of RNA substrate, and various concentrations of ribavirin 5’-triphosphate (50, 100, 250, 300, 500 and 750 μM) in a 50-μl reaction mix (12). The RNA substrate mix was prepared using purified T7 DNA primase in conjunction with the synthetic DNA oligonucleotide Tn,CCTG (10 μM), 300 μM CTP, and 200 μM cap analogue to obtain capped AC5 (GpppAC5 and m7GpppAC5) as described (13). The methyltransferase reaction was incubated at 30 °C and monitored from 30 min to 3 h. 8-μl aliquots were spotted onto DEAE-81 filter paper (Whatman) and washed with 20 mM sodium formate, pH 8.0 to remove any remaining S-adenosylmethionine. The experiment without ribavirin 5’-triphosphate served as a reference. Radioactively labeled RNA was quantified by liquid scintillation counting.

Structure Determination and Refinement—Crystals of NS5MTase,DV were grown by vapor diffusion as described (12) and further soaked for 3 h in 2 mM RTP. A 2.6-A data set was collected at 100 K using a charge-coupled device detector (ADSC Q4) at ID14-2 beamline, ESRF, Grenoble, France. Data were processed using DENZO (14), and intensities were merged with SCALA (15). Since the unit cell dimensions of the crystal were slightly different from those of the apo-form, the structure was solved with the molecular replacement program AmoRe, whereas polyethylene chain of the apo-form served as a model (PDB code 1L9K). A random set comprising 5% of the data was omitted from refinement for Rmerge calculation (16). A round of simulated annealing refinement, as implemented using crystallography CNS software, was performed (17). As in the case of the apo-form, no density was observed for the least favorable residues. Models for one molecule of S-adenosyl-l-homocysteine (SAHC) and one molecule of ribavirin 5’-monophosphate were built into the Fv − F̅ and 2Fv − Fv SIGMA-weighted electron density. The model was further built and refined through alternating cycles using the programs TURBO-FRODO (18) and CNS (crystallography NMR software), respectively. During this process seven sulfate ions, which were already present in the native structure, and 66 additional water molecules were refined. Refinement statistics are given in Table I. Atomic coordinates have been deposited in the Protein Data Bank under code 1R6A.

RESULTS

Inhibition of the RNA 2'-O-Methyltransferase Activity of Dengue Virus NS5MTase,DV Domain—The NS5MTase,DV domain is able to bind GTP (12). In addition, the crystal structure of a ternary complex consisting of NS5MTase,DV, SAHC, and GTP has been described previously (12). In this complex, GTP mimics an RNA cap. Since NS5MTase,DV acts as an RNA 2'-O-methyltransferase (12), we tested whether RTP could have a direct effect on this activity. Since ribavirin is a guanosine analogue and a weak inhibitor of flavivirus growth (7–9, 19, 20), the NS5MTase,DV domain may be a target for RTP. In the RNA 2'-O-methyltransferase assay, a short capped RNA is incubated with NS5MTase,DV, the radiolabeled methyl donor S-adenosyl-l-methionine, and various concentrations of RTP. The methylated RNA product is monitored over time using a filter paper binding assay (12). Fig. 1A shows the results of such an inhibition assay. RTP inhibits the RNA 2'-O-methyltransferase activity. Under the experimental conditions tested here, the inhibitory concentration required to yield a 50% inhibition (IC50) is 101 ± 20 μM, leading to the conclusion that RTP is indeed an inhibitor of the RNA 2'-O-methyltransferase activity contained in the NS5MTase,DV domain.

Mapping of the Guanosine Analogue Binding Site to the Dengue Virus NS5MTase,DV Domain—The crystal structure of NS5MTase,DV (12) indicates that this domain has two nucleoside/nucleotide binding pockets (i.e. one for SAHC and the other for GTP), which could account for the binding of RTP and subsequent inhibition of the RNA 2'-O-methyltransferase activity. Since RTP is a GTP analogue, a GTP-binding competition experiment was used to map the RTP binding site. When the protein is incubated with a fixed concentration of radiolabeled GTP and increasing concentrations of RTP, GTP binding is decreased (Fig. 1B). This indicates that RTP displaces GTP from NS5MTase,DV. When the relative inhibition is plotted against RTP concentration, an apparent Kd value of 55 ± 9 μM is determined. RTP exhibits a NS5MTase,DV binding affinity similar to that of GTP (Kd = 58 ± 14 μM (12)). We then tested the binding of other analogues to NS5MTase,DV. Acyclovir 5’-triphosphate is a guanosine analogue with an acyclic backbone replacing the ribose, and EICAR 5’-triphosphate is a 5-deaza 5-ethynyl ribavirin 5’-triphosphate. Both analogues bound to NS5MTase,DV with Kd values of 80 ± 7 and 165 ± 7 μM, respectively (Fig. 1C). Finding that RTP has the best affinity of the three guanosine analogues and that the acyclic moiety of acyclovir 5'-TP is seemingly less important for binding than a modified base such as that of EICAR prompted us to assay other RTP analogues modified on their ribose moiety (6). A
FIG. 1. Inhibition of the RNA 2'-O-methyltransferase activity of NS5MTase<sub>DV</sub> and mapping of the binding site of guanosine analogues. A, inhibition of RNA 2'-O-methyltransferase activity. The incorporation of H-labeled methyl groups into small capped RNAs was performed using a filter paper binding assay as described under “Experimental Procedures” and in Ref. 12. RTP was added at indicated concentrations immediately prior to the addition of purified NS5MTase<sub>DV</sub> to start the reaction. The plot is an average of three independent experiments. B, determination of the dissociation constant (K<sub>d</sub>) of RTP for NS5MTase<sub>DV</sub>. The protein was incubated with a constant concentration of [32P]GTP (58 μM, the apparent K<sub>d</sub> concentration) and increasing concentrations of RTP. The mixture was irradiated with UV light and analyzed using denaturing gel electrophoresis as described under “Experimental Procedures.” The upper panel shows the Coomassie Blue-stained gel, and the lower panel depicts the corresponding autoradiographic analysis. Quantitation of the autoradiographic analysis is plotted, and the relative inhibition of GTP-binding as a function of RTP concentration is shown. Curve fitting was performed with a hyperbolic equation. C, same analysis as in B using acyclovir-5'TP and EICAR 5'TP. After the autoradiograms were analyzed and quantified, the data were plotted as inhibition of GTP binding as a function of acyclovir-5'TP (upper plot) or EICAR 5'TP (lower plot) concentration.
Inhibition of Flavivirus 2′-O-MTase by Ribavirin 5′-TP

In this paper, we have show that RTP inhibits the RNA 2′-O-methyltransferase activity of dengue virus NS5MTaseDV domain. We further mapped the binding site of RTP binding to the GTP/RNA cap binding site using RTP competition experiments. The RTP binding site was then characterized at the atomic level using x-ray crystallography at 2.6 Å resolution.

The structure presented here is the first example of a viral enzyme complexed to a ribavirin nucleotide. Two cellular protein structures have been solved in complex with ribavirin nucleotides: the H122G mutant of nucleoside diphosphate kinase from Dityctostelea discoideum (PDB code 1NM9 (6)) and the inosine-5′-monophosphate dehydrogenase from Tritrichomonas foetus (PDB code 1ME8 (5)). In the former complex, the binding of RTP is completely aspecific; the phosphate and ribose of RTP interact via hydrogen bonding with the protein, but the only ribavirin pseudobase-protein interaction is an aromatic stacking involving Phe-64, which could be achieved by any nucleobase (Fig. 3, A and E). The atomic contacts are different in the complex of RMP with inosine-5′-monophosphate dehydrogenase, as the ribavirin pseudobase establishes three hydrogen bonds with the protein (Fig. 3F). In the ternary complex with coenzyme A (PDB code 1ME7), no structural rearrangements take place, as the CoA binds in a preformed binding site and the interactions of ribavirin with the protein are not modified. However, a comparison of the relative position of RMP and IMP in complex with inosine 5′-monophosphate dehydrogenase reveals that ribavirin superimposes with IMP perfectly, contrary to what is observed in the NS5MTaseDV-RTP complex. This is because of the intrinsic nature of both nucleotides; with IMP, the NH2 group of ribavirin could have been mimicked by the NH group in the 1-position, whereas with GMP, either the 1- or the 2-position could have been mimicked by the NH2 group of ribavirin.

It is not known whether ribavirin targets NS5MTaseDV in flavivirus-infected cells. It has been demonstrated that ribavirin significantly reduces the growth of several flaviviruses, with no information about the actual viral target (7–9, 19, 20). In the case of dengue serotypes 1, 2, and 3, West Nile, and yellow fever viruses, the reported I50 values are between 81 and 171 μM (7, 9, 20). The apparent inhibition parameters observed in this report for RTP (IC50 ~100 μM) are in good agreement with these I50 values. Because ribavirin decreases the intracellular GTP concentration drastically, it potentiates the successful competition of RTP against GTP for NS5MTaseDV binding. Indeed, the average intracellular GTP concentrations in various mammalian cell types have been
FIG. 3. Structural features of the nucleotide binding site of NS5MTaseDV. A, the model of GDPMP in the nucleotide binding site of NS5MTaseDV has been described in Ref. 12. Residues interacting with the nucleotide are shown in ball-and-stick form. Main-chain carbon atoms are shown in dark blue except for the carbonyl oxygens, which are red. Side chains are colored according to atom type. For clarity, noninteracting side chains of residues 17, 19, and 20 are not shown. Dotted lines indicate hydrogen bonds. B, 2\(F_o - F\) electron density map of the refined complex between RTP and NS5MTaseDV. The color code is the same as in A. The \(\beta\)- and \(\gamma\)-phosphates of RTP are present but could not be modeled accurately because of poor electron density in this area, suggesting the coexistence of several conformations. Schematic diagrams show the interactions of the guanine moiety of GDPMP (C) and the ribavirin pseudobase with NS5MTaseDV residues (D). Main-chain carbonyl oxygens within hydrogen bonding distance (in \(\text{Å}\)) of the GDPMP and RTP 2-amino group are indicated by dotted lines (only two of the three displayed H-bonds can be fulfilled simultaneously). "O - C" symbolizes main chain carbonyl groups. E, superimposition of ribavirin triphosphate and GDPMP as seen in the NS5MTaseDV domain. The structures of the two complexes (NS5MTaseDV-GDPMP-SAHC and NS5MTaseDV-RTP-SAHC) have been superimposed. For clarity, only a few amino acids from the binding site are shown in stick representation (K14, L17, N18, A19, L20). The main-chain carbon atoms are shown in dark blue, except for the carbonyl oxygens of Leu-17, Asn-18, and Leu-20 (which are within hydrogen bond distance of the NH\(_2\) group of the guanosine base and the ribavirin pseudobase; see A and B) and for the amino group of Lys-14 (which interacts via hydrogen bonding with the O-2 position of the ribose; see A and B). GDPMP is also represented in dark blue, except for oxygen and nitrogen atoms. Ribavirin triphosphate is represented in ball-and-stick form and colored according to atom type. F, schematic diagram showing the interactions of the ribavirin pseudobase with inosine 5'-monophosphate dehydrogenase (PDB code 1MES). Dotted lines indicate hydrogen bonds, with numbers corresponding to distance in Å.
measured and found to be ~0.48 μM (compiled in Ref. 21). Treatment of murine leukemia L1210 cells with 20 μM ribavirin decreases the GTP concentration to a value equal to 12% of that of the untreated control, i.e., around 60 μM (22). This latter value is precisely the same as that of the \( K_D \) (GTP) for NS5MTase\(_{DV} \) (58 ± 14 μM (12)). This observation suggests that with ribavirin treatment, which involves ribavirin concentrations of 600–800 μM in biological fluids (23), the down-regulated GTP concentration is not likely to saturate NS5MTase\(_{DV} \), leaving open the possibility that the binding of RTP to NS5MTase\(_{DV} \) may be relevant to the known ribavirin antiviral activity. This discussion may also be relevant to other viruses. Indeed, RTP has been reported to be an inhibitor of the (guanine-N7)-methyltransferase of vaccinia virus, as well as to lead to abnormal RNA cap formation in alphaviruses (reviewed in Ref. 24). To date, there is little evidence that ribavirin actually targets viral enzymes other than RNA polymerases, as in the case of hepacviruses (3, 25). The structural data presented here suggest a model for the inhibition of the RNA 2'-O-methyltransferase activity by RTP. RTP may compete with viral RNA cap structures to bind the NS5MTase\(_{DV} \) and prevent efficient RNA cap methylation.

Whether or not RTP causes flavivirus growth inhibition through the targeting of NS5MTase\(_{DV} \) in vitro, our results still have implications in terms of drug design. First, screening of the Protein Data Bank revealed that no specific recognition of GTP with only the purine 2-position (as shown in Fig. 3, B and D) has been reported before that of NS5MTase\(_{DV} \) (12). The great majority of cellular NTP-binding enzymes appear to contact at least two of the purine at position 1, 2, or 6 (12). Among over 60 protein-GTP or protein-RNA cap complexes examined, only two (PDB codes 1KHB and 1DOA) bind GTP using a single position (O-6 in this case) in addition to base-stacking contacts. In theory, since RTP and GTP have equivalent H-bond acceptors and acceptors at positions 1 and 6 but not position 2, RTP could bind to GTP-binding proteins using positions 1 and 6. To the best of our knowledge, there is no example of such binding in the Protein Data Bank involving a protein of human origin. The observation of an antiviral selectivity for ribavirin may indicate that ribavirin either targets viral proteins through this position 2 alone (amino of RTP) or binds both viral and cellular GTP-binding proteins through positions 1 and 6 (carbonyl and the NH of RTP) but has a more profound effect on viral targets, hence the observed selectivity. Second, the same series of RTP analogues has been used to inhibit HCV NS5B, a hepacivirus RNA-dependent RNA polymerase (6). Hepacviruses do not have an RNA capping machinery, but their RNA-dependent RNA polymerase domain is ~10% identical (22% amino acid similarity) to that of flaviviruses. We note that the most important ribose groups for binding/inhibition are not similar between the hepatitis C virus NS5B polymerase and NS5MTase\(_{DV} \). For hepatitis C virus NS5B the 3'-hydroxyl is critical (6), whereas our present results indicate that the 2'-hydroxyl is most important in the case of NS5MTase\(_{DV} \). Therefore, if the most important group is also the 3'-hydroxyl for the flavivirus RNA polymerase, it seems difficult to design a simple ribose modification that would target both the RNA polymerase and the cap-binding enzyme of flaviviruses simultaneously. Third, only main-chain contacts are involved in the binding of RTP to NS5MTase\(_{DV} \). A mere substitution of an amino acid side chain cannot directly discriminate RTP relative to GTP in order to provide potential drug resistance.

Since RNA capping is essential for various viruses (18), the mechanism for ribavirin-mediated inhibition of RNA capping presented here may account in part for the antiviral activity of ribavirin against flaviviruses, but it does not exclude the inhibition of additional viral enzymatic activities. Our results would then provide a basis for rational drug design against human pathogens of viral origin, of which the emerging flaviviruses are a timely example.

Acknowledgments—We thank Sonia Longhi, Zhi Hong, Cindy Yee, and Holli Conway for critical reading of the manuscript.

REFERENCES

1. Sidwell, R. W., Huffman, J. H., Khare, G. P., Allen, L. B., Witkowski, J. T., and Robinson, B. K. (1972) Science 177, 705–706.

2. Patterson, J. L., and Fernandez-Larsson, R. (1990) Rev. Infect. Dis. 12, 1139–1146.

3. Hong, Z., and Cameron, C. E. (2002) J. Virol. 76, 41–69.

4. Crotty, S., Maag, D., Arnold, J. J., Zhong, W., Lau, J. Y., Hong, Z., Andino, R., and Cameron, C. E. (2000) Nat. Med. 6, 1375–1379.

5. Prestoe, G. L., Wu, J. Z., and Luetteke, H. (2002) J. Biol. Chem. 277, 50654–50659.

6. Gallais-Montbrun, S., Chen, Y., Dutruelle, H., Sephys, M., Morera, S., Guérin, C., Schneider, E., Mulard, L., Janin, J., Veron, J. M., Deville-Bonne, D., and Canard, B. (2003) Mol. Pharmacol. 63, 538–546.

7. Neys, J., Meerbach, A., McKenna, P., and De Clercq, E. (1996) Antiviral Res. 30, 125–132.

8. Jordan, I., Briese, T., Fischer, N., Lau, J. Y., and Lipkin, W. I. (2000) J. Infect. Dis. 182, 1214–1217.

9. Crance, J. M., Scaramozzino, N., Jouan, A., and Garin, D. (2003) Antiviral Res. 58, 75–79.

10. Anderson, J. F., Andreadis, T. G., Vossbrinck, C. R., Tirrell, S., Wakem, E. M., French, R. A., Garmoneda, A. E., and Van Kruiningen, H. J. (1999) Science 286, 2331–2333.

11. Chambers, T. J., Hahn, C. S., Galler, R., and Rice, C. M. (1999) Annu. Rev. Microbiol. 44, 649–668.

12. Egloff, M. P., Benaroch, D., Selisko, B., Romette, J. L., and Canard, B. (2002) EMBO J. 21, 2757–2768.

13. Matsuo, H., Moriguchi, T., Tagaki, T., Kasakabe, T., Buratowski, S., Sekine, M., Kyogoku, Y., and Wagner, G. (2000) J. Am. Chem. Soc. 122, 2417–2421.

14. Gnosowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326.

15. CCP4. (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763.

16. Brunger, A. T. (1992) Nature 355, 472–474.

17. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gus, P., Grossenbacher, R. W., Jiang, J. S., Kuzevsky, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 965–971.

18. Roussell, A., and Cambillau, C. (1991) Silicon Graphics Directory, p. 97, Mountain View, CA.

19. Keef, W. C., Elm, J. L., Jr., and Halstead, S. B. (1982) Antiviral Res. 2, 69–79.

20. Leyssen, P., Van Lommel, A., Drosten, C., Schmitz, H., De Clercq, E., and Neys, J. (2001) Virology 297, 27–37.

21. Kernberg, A., and Baker, T. (1992) DNA Replication 2nd Ed., p. 41, W. H. Freeman, New York.

22. Balzarini, J., Karlsson, A., Wang, L., Bohman, C., Horzka, K., Votruba, I., Fridland, A., Van Aerschot, A., Herdwijn, P., and De Clercq, E. (1993) J. Biol. Chem. 268, 24593–24598.

23. Gilbert, B. E., and Knight, V. (1986) Antimicrob. Agents Chemother. 30, 201–205.

24. Bisaiollion, M., and Lemaig, G. (1997) Virology 256, 1–7.

25. Young, K. C., Lindsay, K. L., Lee, K. J., Liu, W. C., He, J. W., Milstein, S. L., and Lai, M. M. (2003) Hepatology 38, 869–878.