Interactions of a Subassembly of the Herpes Simplex Virus Type 1 Helicase-Primase with DNA*  

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The UL5, UL8, and UL52 genes of herpes simplex virus type 1 encode a multisubunit assembly that possesses primase, DNA helicase, and DNA-dependent nucleoside triphosphatase activities. A subassembly consisting of the UL5 and UL52 gene products retains these activities. The nucleoside triphosphatase activity of the UL5/UL52 subassembly is strongly stimulated by both homo- and heteropolymeric single-stranded DNA. Double-stranded DNA has little ability to stimulate the ATPase activity. The subassembly binds both double and single-stranded DNA. Nucleotides are not required for DNA-binding. The minimum length of single-stranded DNA that is bound and that stimulates enzymatic activity is about 12 nucleotides. The kinetic parameters of the ATPase activity of the subassembly are affected by the length of the oligonucleotide coeffectors. The K_m decreases as the coeffector length is increased up to a length of about 20 nucleotides and then remains independent of coeffector length. The first order rate constant for ATPase activity exhibits a quasihyperbolic dependence on the length of the DNA coeffector and is maximal for coeffectors of 20 nucleotides and longer.

Herpes simplex virus type 1 (HSV-1) encodes a heterotrimeric DNA helicase-primase whose subunits are encoded by the UL5, UL8, and UL52 genes of the virus (1, 2). The helicase activity of the enzyme is coupled to the hydrolysis of either ATP or GTP (1, 3). The UL5 gene product possesses a set of domains that correspond with several conserved motifs found in other DNA helicases (4). Mutagenesis of any one of these conserved domains in UL5 protein obliterates viral DNA synthesis (5). The UL52 gene is also essential for viral DNA synthesis and encodes a conserved domain that is found in other primases (6–8). Mutagenesis of this conserved domain specifically obliterates the primase activity of the HSV-1 helicase-primase (7, 8). Deletion of the UL8 gene from HSV-1 renders the virus incapable of DNA replication (9).

The HSV-1 helicase-primase can be isolated from insect cells that have been simultaneously infected with recombinant baculoviruses that express each of the three subunits that compose the holoenzyme (10). A subassembly consisting of the UL5 and UL52 gene products also exhibits the DNA-dependent ATPase, DNA helicase, and primase activities that are associated with the holoenzyme (11, 12). The primase activity of this subassembly exhibits DNA sequence dependence and is stimulated by the UL8 protein (13–15). However, purified UL8 protein itself lacks any type of discernable enzymatic activity (11, 12).

Homologs of the genes encoding the HSV-1 helicase-primase are found in several other human herpesviruses (16). The complex array of activities possessed by the helicase-primases encoded by this group of viruses may be attractive targets for antiviral drug design. An understanding of the properties of the HSV-1 helicase-primase may lead to strategies for developing such compounds.

In this work we further examine the interaction of the UL5/UL52 subassembly with various DNA coeffectors. Although the enzyme binds both double- and single-stranded DNA, double-stranded DNA fails to stimulate the NTPase activity of the enzyme to the same extent as does single-stranded DNA. Nucleotides are not required for DNA binding. Evidence is presented that shows that activation of NTPase activity varies with the length of the DNA coeffector and that the minimal DNA-binding site is about 12 nucleotides in length. The kinetic parameters for the ATPase activity of the subassembly are also affected by the length of the oligonucleotide coeffector and suggest that the full-length binding site is at least 20 nucleotides.

EXPERIMENTAL PROCEDURES

Materials—Deoxythymidine oligonucleotides were obtained from U.S. Biochemical Corp. or Midland Certified Reagent Co. (Midland, TX). The plasmid pVL941 (17) and M13mp18 single-stranded DNA were purified according to standard procedures (18). Nucleotides for the NTPase assays were from Pharmacia Biotech Inc. Nucleotides for the filter-binding assays were from Sigma. Bovine serum albumin fraction V was from Sigma. Malachite green hydrochloride and ammonium molybdate tetrahydrate, used in the colorimetric DNA-dependent ATPase assay, were from Sigma and Aldrich, respectively. Buffers used for the filter-binding assay were from Schleicher & Schuell. (γ-32P)ATP was from Amersham Corp. T4 polynucleotide kinase was from Life Technologies, Inc. Hydroxylapatite (Bio-Gel HTP) was from Bio-Rad. The UL5/UL52 subassembly was purified as described (12) except that hydroxylapatite fractionation was used in place of the gel filtration step. Pooled fractions containing the enzyme were loaded onto hydroxylapatite (1 mg of protein/ml of bed volume) and eluted with a 10–400 mM sodium phosphate buffer gradient, pH 7.5, containing 10% glycerol, 2 mM dithiothreitol, 4 mM MgCl_2, 2 mM dithiothreitol, and 4 mM leupeptin. Fractions containing the enzyme were pooled, diluted 4-fold in buffer containing 25 mM Tris-HCl, pH 8.0, 10% glycerol, 2 mM dithiothreitol, 2 mM EDTA, 2 mM EGTA, 4 μg/ml peptatin, and 4 μg/ml leupeptin and applied to a 0.5-m1 column of DEAE. The column was then washed, and the enzyme was step-eluted with equilibration buffer supplemented with 250 mM NaCl.

DNA-dependent NTPase Assay—NTPase assays were essentially performed as described (1). Reaction mixtures (25 μl) contained 25 mM HEPES, pH 7.5, 3.5 mM MgCl_2, 2.5 mM ATP, 10% glycerol, 2 mM dithiothreitol, 100 μg/ml bovine serum albumin, and the indicated amounts of DNA and the UL5/UL52 subassembly. For experiments in

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1 The abbreviations used are: HSV-1, herpes simplex virus type 1; AMPPNP/NH_2, β,γ-imidodoenosine 5′-triphosphate; EPPS, N,2-hydroxyethylpiperazine-N′-3-propanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; TAPS, 3-tris(hydroxymethyl)methylaminopropanesulfonic acid.
which the pH was varied, the following buffers were used in place of HEPES: PIPES, pH 6.5 and 7.0; EPPS, pH 8.0; TAPS, pH 8.5 and 9.0. Incubations were carried out at 37°C for 30 min and then terminated by the addition of 0.75 ml of acidic molybdenum. Five minutes after termination, the absorbance at 650 nm was determined. The amount of inorganic phosphate that was released was determined by comparison with a standard curve in which 1 nmol of phosphate had an absorbance of 0.1.

**Determination of Kinetic Parameters for ATPase Activity**—The NT-Pase assay described above was used with the following modifications. Reaction mixtures contained 32 μM UL5/UL52, 30 μM oligonucleotide, and varying concentrations of ATP. The mixtures were incubated for 12 min at 34°C and then terminated and developed as described above. The program DeltaGraph was used to calculate the $K_m$ and $V_{max}$ values from data that were directly fit to the Michaelis-Menten equation by nonlinear regression.

**Filter Binding Assay**—Nitrocellulose filter binding was performed using the double filter method of Wong and Lohman (19). The 9.8-kilobase plasmid pVLJ41 was cleaved into 10 blunt-ended fragments with HindII restriction enzyme. The fragments ranged in length from 27 to about 4,600 base pairs. The fragments were end-labeled using [γ-32P]ATP and T4 kinase using standard procedures (18). Oligo(dT) ranging in length from 8 to 20 nucleotides was end-labeled similarly.

The effect of pH on the DNA-dependent ATPase is shown in Fig. 1. The optimal pH was 7.5, and it dropped abruptly on either side of the optimum. This result is similar to that reported for the holoenzyme (20). Recent evidence suggests that the HSV-1 helicase-primase has two distinct NT-Pase sites (3). One site hydrolyzes ATP only, and the other site hydrolyzes both GTP and ATP. We therefore also examined the effect of pH on the GTPase activity of the enzyme (Fig. 1). The pH profile for GTPase activity was the same as that for ATPase. Thus, if there are two NT-Pase sites, their pH profiles are similar.

The effect of Mg$^{2+}$ and various salts on the DNA-dependent ATPase activity of the UL5/UL52 subassembly was also examined. The optimal Mg$^{2+}$ concentration for the DNA-dependent ATPase activity ranged from about 1 to 4 mM (Fig. 1b). These optimal Mg$^{2+}$ concentrations are the same as those reported for the holoenzyme (20).

Different salts inhibited the DNA-dependent ATPase activity in a concentration-dependent manner (Fig. 1c). Eighty percent of maximal activity was observed in the presence of 50 mM acetate and chloride salts. At higher salt concentrations the chloride ion was more inhibitory than was acetate. The sulfate ion abolished activity at a concentration of 50 mM.

**Optimization of Reaction Conditions for the DNA-dependent ATPase Activity of the UL5/UL52 Subassembly**—In order to examine the interaction of the UL5/UL52 subassembly with nucleic acids we first sought to identify pH and salt conditions that would yield maximal DNA-dependent ATPase activity. We also wanted to ascertain that conditions optimal for the ATPase activity of the subassembly are the same as those reported for the holoenzyme (20). We assumed that conditions that are optimal for the DNA-dependent ATPase activity of the enzyme would be optimal for interactions with nucleic acids.

The effect of pH on the DNA-dependent ATPase is shown in Fig. 1a. The optimal pH was 7.5, and it dropped abruptly on either side of the optimum. This result is similar to that reported for the holoenzyme (20). Recent evidence suggests that the HSV-1 helicase-primase has two distinct NT-Pase sites (3). One site hydrolyzes ATP only, and the other site hydrolyzes both GTP and ATP. We therefore also examined the effect of pH on the GTPase activity of the enzyme to determine if the two sites could be distinguished by differences in their pH optima (Fig. 1a). The pH profile for GTPase activity was the same as that for ATPase. Thus, if there are two NT-Pase sites, their pH profiles are similar.

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DNA (24). Other helicases, such as the first bind to a specific nucleotide substrate before binding to origin-binding protein.

Poly(dT) and single-stranded M13mp18 DNA were equally effective, however, poly(dA) was about 4-fold less effective as an activator. ATPase activity of the UL5/UL52 subassembly (Fig. 2 (21–23). Both poly(dT) and poly(dC) effectively activated the ATPase activity of the UL5/UL52 subassembly. Heteropolymeric DNA is much less effective than homopolymeric DNA in stimulating the DNA-dependent ATPase—

we next compared the effects of homopolymeric and heteropolymeric single-stranded DNA on the ATPase activity of the UL5/UL52 subassembly. Heteropolymeric DNA is much less effective than homopolymeric DNA in stimulating the DNA-dependent ATPase activity of the HSV-1 origin-binding protein, which is also a DNA helicase and is encoded by the UL9 gene (21–23). Both poly(dT) and poly(dC) effectively activated the ATPase activity of the UL5/UL52 subassembly (Fig. 2b). However, poly(dA) was about 4-fold less effective as an activator. Poly(dT) and single-stranded M13mp18 DNA were equally effective in supporting the ATPase activity of the enzyme (Fig. 2c). This result contrasts with that observed for the HSV-1 origin-binding protein.

The Effect of Nucleotides on DNA Binding—Some helicases, such as the gene 4 protein encoded by bacteriophage T7, must first bind to a specific nucleotide substrate before binding to DNA (24). Other helicases, such as the Escherichia coli Rep helicase, do not require a nucleotide cofactor for DNA binding (25). We used nitrocellulose filter binding to assess the ability of the UL5/UL52 subassembly to bind to single-stranded and double-stranded plasmid DNA fragments in the absence or in the presence of nucleotide. In the absence of nucleotide, the subassembly bound both single- and double-stranded DNA (Fig. 3a). However, the enzyme bound single-stranded DNA about 5-fold more effectively than it did double-stranded DNA.

The effect of nucleotides on DNA binding was examined next. We next sought to determine under initial rate conditions the minimal length of single-stranded DNA that activates the ATPase and GTPase of the UL5/UL52 subassembly. Deoxythymidine oligonucleotides ranging in length from 6 to over 1000 residues were tested for the ability to activate the DNA-dependent ATPase and GTPase activities of the enzyme (Fig. 4, top). The activation profile was similar for both ATPase and GTPase. Little activation of either ATPase or GTPase was observed for oligonucleotides less than 12 residues in length. Significant activation of both ATPase and GTPase was observed when oligo(dT)₁₂ was used as the effector. The extent of activation continued to increase as the length of the oligo(dT) effector was increased and reached an apparent maximum for oligonucleotides between 36 and 60 residues in length for both the ATPase and GTPase activities.

We also used nitrocellulose filter binding to estimate the minimal length of DNA that is bound by the UL5/UL52 subassembly (Fig. 4, bottom). The minimum length that was bound was 10–12 nucleotides. The extent of binding to (dT)$_{16}$ and (dT)$_{20}$ was somewhat higher. Identical results were obtained by gel mobility shift analysis (data not shown). Thus, the minimal length of DNA that is effectively bound and that elicits NTPase activity is about 10–12 nucleotides. We conclude that, if there are two separate DNA binding sites, this activity is exerted through two distinct DNA-bindingsites that separately modulate the activity of the two putative NTPase sites (3). We sought to determine if these two sites could be distinguished based on differences in the size of the DNA binding sites. We assumed that the size of the binding site(s) could be estimated by determining the minimal length of single-stranded DNA that activates the ATPase and GTPase of the UL5/UL52 subassembly. Deoxythymidine oligonucleotides ranging in length from 6 to over 1000 residues were tested for the ability to activate the DNA-dependent ATPase and GTPase activities of the enzyme (Fig. 4, top). The activation profile was similar for both ATPase and GTPase. Little activation of either ATPase or GTPase was observed for oligonucleotides less than 12 residues in length. Significant activation of both ATPase and GTPase was observed when oligo(dT)$_{12}$ was used as the effector. The extent of activation continued to increase as the lengths of the oligo(dT) effector was increased and reached an apparent maximum for oligonucleotides between 36 and 60 residues in length for both the ATPase and GTPase activities.

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Effect of DNA Length on the Kinetic Parameters for ATPase—We next sought to determine under initial rate conditions whether the progressive increase in ATPase activity that occurred with longer DNA coeffectors is exerted through...
In this work we have characterized the interaction of an active subassembly of the HSV-1 helicase-primase with various DNA coeffectors and have assessed their capacity to activate the nucleoside triphosphatase activity of the enzyme. We initiated this study by establishing conditions that maximize the NTPase activity of the UL5/UL52 subassembly. We assume that the pH, Mg\(^{2+}\) and salt optima for NTPase activity are also optimal for the binding of DNA coeffectors by the enzyme. The enhancement of the hydrolytic efficiency or through the enhanced binding of the ATP substrate by the enzyme. The ATPase activity of the UL5/UL52 subassembly was assayed in the presence of constant concentrations of oligo(dT)\(_{n}\) of increasing lengths while the ATP concentration was varied. The \(V_{\text{max}}\) and \(K_m\) were then determined and plotted as a function of the length of the oligo(dT) activator (Fig. 5, a and b). \(V_{\text{max}}\) appeared to increase in a roughly linear manner as the length of the oligonucleotide increased (Fig. 5a). However, the \(K_m\) for ATP was constant for oligo(dT) containing 20 or more residues (Fig. 5b). The \(K_m\) for ATP was higher in the presence of oligo(dT) of lengths shorter than 20 nucleotides. The effect of the length of the DNA coactivator on the first order rate constants (\(V_{\text{max}}/K_m\)) showed a quasihyperbolic effect (Fig. 5c). The first order constant appeared to reach a maximum for DNA coactivators between 20 and 30 nucleotides in length and could be extrapolated to a minimum of about 10 nucleotides in length. These results suggest that the affinity for ATP is progressively enhanced up to a critical length of the DNA coactivator and then remains constant while the efficiency of catalysis also increases and reaches a maximum once the DNA binding site is fully occupied.

DISCUSSION

In this work we have characterized the interaction of an active subassembly of the HSV-1 helicase-primase with various DNA coeffectors and have assessed their capacity to activate the nucleoside triphosphatase activity of the enzyme. We initiated this study by establishing conditions that maximize the NTPase activity of the UL5/UL52 subassembly. We assume that the pH, Mg\(^{2+}\) and salt optima for NTPase activity are also optimal for the binding of DNA coeffectors by the enzyme. The pH and Mg\(^{2+}\) conditions that we identified as optimal for the DNA-dependent NTPase activity of UL5/UL52 subassembly were the same as those reported for the holoenzyme (20). The range of pH that supports maximal DNA-dependent ATPase and GTPase is identical, with an optimum pH of 7.5. The pH optimum of the helicase-primase contrasts significantly with that of the UL9 protein, which is another HSV-1-encoded DNA helicase that is required for viral replication and has a pH optimum between 8.5 and 9.0 (23).

Kinetic evidence suggests that the HSV-1 helicase-primase has two sites that hydrolyze nucleoside triphosphates in a DNA-dependent manner. Both of these sites can apparently drive the unwinding of duplex DNA (3). The UL5 gene product contains a consensus nucleotide binding site near its amino terminus (27, 28). The location of the second putative NTP binding site on the enzyme is not known. It could reside either on the UL5 or the UL52 gene product. This second site would differ in structure from the consensus sequence at the UL5 N terminus and thus might have a different pH profile. Our failure to detect differences in the pH profiles for the DNA-dependent ATPase and GTPase activities of the enzyme suggests that there is either one DNA-dependent NTPase site, or, if there are two NTPase sites, their pH optima are similar.

The effect of nucleic acid composition and structure on the NTPase activity of the UL5/UL52 subassembly also differs from that of the UL9 protein. Double-stranded DNA functions poorly as a coactivator for the UL5/UL52 subassembly. However, double-stranded DNA containing specific sequences from the HSV-1 origin of replication are capable of eliciting the ATPase activity of the UL9 gene product. Single-stranded DNA is much more efficient than double-stranded DNA in stimulating the ATPase activity of the UL5/UL52 subassembly. Our obser-

M. S. Dodson and I. R. Lehman, unpublished results.
vation that homopolymeric RNA is essentially inert as a cofactor suggests that recognition of the sugar-phosphate backbone is crucial for binding or for translocation of the enzyme along the DNA. Single-stranded homopolymers containing pyrimidines function just as well as heteropolymeric single-stranded DNA in stimulating the ATPase activity of the UL5/UL52 subassembly. In contrast to the UL9 protein, the HSV-1 helicase-primase utilizes heteropolymeric and homopolymeric single-stranded DNA equally well for activation of ATPase activity. Presumably the UL9 gene product, which functions as an origin-binding protein, is far less effective than the helicase-primase for unwinding regions of secondary structure in single-stranded DNA.骨化作用 furthermore, the DNA-dependent ATPase activity of the UL9 gene product is much weaker than that of the HSV-1 helicase-primase regardless of the type of single-stranded cofactor that is used. These distinctions may reflect different mechanisms of DNA unwinding by these two helicases, both of which have a different role in viral DNA replication.

The DNA binding properties of the UL5/UL52 subassembly differ from those of the helicase-primases encoded by the bacteriophages T4 and T7 (24). In contrast to the enzymes encoded by these phages, the binding of DNA by the herpes-encoded enzyme does not require prior binding of nucleotide. However, the affinity of the enzyme for DNA appears to be modulated by the presence of nucleotides in a manner that may be similar to that observed for the Rep helicase encoded by E. coli (26). Thus, the mechanism of DNA unwinding of the herpes enzyme may differ from the helicase-primases encoded by bacteriophages.

Recent evidence suggests that there are two separate DNA-binding sites on the HSV-1 helicase-primase that activate NTPase activity of the enzyme (3). One of these DNA-binding sites activates the ATPase site that hydrolyzes both GTP and ATP. The second DNA-binding site activates the site that hydrolyzes ATP only. We expected that the sizes of these two DNA-binding sites might differ and could be discriminated by differences in the lengths of poly(dT) that are required for NTPase activation. However, the minimal length of poly(dT) that stimulates the ATPase and GTPase activity of the UL5/UL52 subassembly is the same, about 12 nucleotides. The extent of stimulation for the ATPase and GTPase activities also appears to reach a maximum at about 36 nucleotides, which may reflect complete occupancy of the binding site. Tenney et al. (15) have obtained similar results for the stimulation of ATPase activity with heteropolymeric oligonucleotides. Thus, if there are two DNA-binding sites, the sizes of the binding pockets are similar.

The effect of the length of the DNA cofactor on the kinetic parameters for the ATPase activity of the UL5/UL52 subassembly is complex. The first order rate constant for the ATPase activity of the UL5/UL52 subassembly increases as the length of the DNA cofactor is increased and reaches a plateau for effectors of 20 nucleotides or longer, while the KM for ATP decreases and then remains constant for effectors of 20 nucleotides or longer. These observations could suggest that in the absence of DNA of a critical length the affinity of the helicase-primase for ATP is low. Upon binding DNA of a critical length, the enzyme shifts into a conformation that productively binds ATP. Further increases in the length of the DNA incrementally push the enzyme into a catalytically more efficient conformation until a maximum is reached at 20 nucleotides or longer. Young et al. (29) observed a somewhat similar phenomenon for the gene 41 helicase-primase encoded by bacteriophage T4. The extreme differences in the ability of poly(dT) versus poly(da) to activate the ATPase activity of the helicase-primase suggests that steric variability in the nucleic acid sequence also affects the manner in which the DNA occupies the binding cleft. Consequently, the differences in catalytic efficiency with variable lengths and sequences of DNA may reflect a need for the binding site to be able to accommodate wide ranges of steric variability as the enzyme translocates along the DNA cofactor.

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REFERENCES
1. Crute, J. J., Mocarski, E. S., and Lehman, I. R. (1988) Nucleic Acids Res. 16, 6585–6596
2. Crute, J. J., Tsurumi, T., Zhu, L., Weller, S. K., Olivo, P. D., Chalberg, M. D., Mocarski, E. S., and Lehman, I. R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2186–2189
3. Crute, J. J., Bruckner, R. C., Dodson, M. S., and Lehman, I. R. (1991) J. Biol. Chem. 266, 21252–21256
4. Gorbalenya, A. E., and Koonin, E. (1993) Curr. Opin. Struct. Biol. 3, 419–429
5. Zhu, L., and Weller, S. K. (1992) J. Virol. 66, 469–479
6. Goldstein, D. J., and Weller, S. K. (1988) J. Virol. 62, 2970–2977
7. Klinedinst, D. K., and Chalberg, M. D. (1994) J. Virol. 68, 3695–3701
8. Dracheva, S., Koonin, E. V., and Crute, J. J. (1995) J. Biol. Chem. 270, 14148–14153
9. Carmichael, E. P., and Weller, S. K. (1989) J. Virol. 63, 591–599
10. Dodson, M. S., Crute, J. J., Bruckner, R. C., and Lehman, I. R. (1989) J. Biol. Chem. 264, 20835–20838
11. Calder, J. M., and Stow, N. D. (1990) Nucleic Acids Res. 18, 5373–5378
12. Dodson, M. S., and Lehman, I. R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1105–1109
13. Sherman, G., Gottlieb, J., and Chalberg, M. D. (1992) J. Virol. 66, 4884–4892
14. Tenney, D. J., Hurlburt, W. W., Micheletti, P. A., Bifano, M., and Hamatake, R. K. (1994) J. Biol. Chem. 269, 5303–5305
15. Tenney, D. J., Sheaffer, A. K., Hurlburt, W. W., Bifano, M., and Hamatake, R. K. (1995) J. Biol. Chem. 270, 9129–9136
16. McGeoch, D. J., Dalrymple, M. A., Davison, A. J., Dolan, A., Frame, M. C., McNab, D., Perry, L. J., Scott, J. E., and Taylor, P. (1988) J. Gen. Virol. 69, 1531–1574
17. Lukow, V. A., and Summers, M. D. (1989) Virology 170, 31–39
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Wong, L., and Lohman, T. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5428–5432
20. Crute, J. J., and Lehman, I. R. (1991) J. Biol. Chem. 266, 4484–4488
21. handmade, N. A., and Chalberg, M. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5414–5418
22. Bruckner, R. C., Crute, J. J., Dodson, M. S., and Lehman, I. R. (1991) J. Biol. Chem. 266, 2669–2674
23. Dodson, M. S., and Lehman, I. R. (1993) J. Biol. Chem. 268, 1213–1219
24. Matson, S. W., and Richardson, C. C. (1985) J. Biol. Chem. 260, 2211–2217
25. Lehman, T. M. (1993) J. Biol. Chem. 268, 2269–2272
26. Wong, L., and Lehman, T. M. (1992) Science 256, 350–355
27. Gorbalenya, A. E., Koonin, E. V., Donchenko, A. P., and Blinov, V. M. (1988) FEBS Lett. 235, 16–24
28. McGeoch, D. J., Dalrymple, M. A., Dolan, A., McNab, D., Perry, L. J., Taylor, P., and Chalberg, M. D. (1988) J. Virol. 62, 444–453
29. Young, M. C., Schultz, D. E., Ring, D., and von Hippel, P. H. (1994) J. Mol. Biol. 235, 1447–1458