Human Cytomegalovirus UL84 Is a Phosphoprotein That Exhibits UTPase Activity and Is a Putative Member of the DE/D/H Box Family of Proteins*

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Human cytomegalovirus (HCMV) UL84 is required for lytic DNA replication and is proposed to be the key factor in initiation of viral DNA synthesis. We now show that UL84 has a high degree of homology to the DExD/H (where x can be any amino acid) box family of helicases, displays UTPase activity, and is phosphorylated at serine residues. Affinity column-purified UL84-FLAG fusion protein was used in an in vitro nucleoside triphosphatase (NTPase) assay to show that UL84 has NTPase activity, preferring UTP. This UTPase activity was linear with respect to enzyme concentration and slightly enhanced by the addition of nucleic acid substrates. UL84 UTPase was the highest at low salt concentrations, a pH of 7.5, and a temperature of 45 °C. The enzyme preferred Mg2+ as the divalent cation but was also able to catalyze the UTPase reaction in the presence of Mn2+, Ca2+, and Zn2+ albeit at lower levels. The evidence presented here suggests that the UL84 UTPase activity may be part of an energy-generating system for helicase activity associated with the initiation of HCMV DNA replication.

DNA through the binding and hydrolysis of NTPs such that the remaining DNA replication machinery can assemble and subsequently carry out DNA synthesis. In other herpesvirus systems, initiator proteins were shown to have properties associated with DNA metabolism. For example, herpes simplex virus type 1 (HSV-1) UL9 exhibits ATPase and helicase activity (2–4). The enzyme is phosphorylated by cellular kinases and exists as a dimer in its active form (5–7). UL9 interacts with Ori4 and Ori5, with the assistance of ssDNA binding protein (ICP8) in a two-step cooperative binding process to initiate DNA replication (8). UL9 also interacts with other components of the DNA replication machinery and may lead to the recruitment of these factors to the site of DNA synthesis (9–11). For Epstein-Barr virus (EBV), Zta was shown to interact with the helicase-primase proteins and is directed to the nucleus via the interaction with the helicase component (12, 13). In addition, Zta is also a multifunctional phosphoprotein, having transcriptional activation properties with respect to up-regulating the expression of viral genes, as well as having a role in initiation of DNA synthesis (14–17). Other DNA virus systems that use a method similar to that of HSV-1 of initiation of DNA replication include polyoma and papillomaviruses. The large T-antigen of SV40 virus has both RNA and DNA helicase activities that are in turn associated with both a non-ATP NTPase activity and ATPase activity, respectively (18). The papillomavirus protein E1 also exhibits ATPase and helicase activity in vitro (19). Both of these initiator proteins require a double hexamer phosphoprotein structure, which surround the DNA substrate to unwind the DNA or RNA strands (20–24).

UL84 is a nuclear protein that interacts with the immediate-early protein IE2 of HCMV and regulates the transactivation activity of IE2 by possibly interfering with IE2 binding to its DNA substrates (25–27). UL84 is an oligomer in vivo, and the point of this self-interaction is located in a highly charged region of the protein, which is distinct from the leucine zipper that is required for IE2-UL84 interaction (27).

IE2 also has a role in DNA replication, since it was demonstrated that UL84 and IE2 cooperate to activate a strong promoter within HCMV oriLyt (28). This oriLyt promoter region can be replaced with the SV40 promoter, alleviating the need for IE2 in the transient assay (28).

Despite being implicated in DNA replication, to date no enzymatic function has been reported for UL84. Amino acid sequence analysis revealed that UL84 has a high degree of identity to the family of proteins called DExD/H box proteins. These proteins were identified as enzymes that utilize the energy of NTPs to promote RNA metabolism including an RNA-RNA helicase, RNA-DNA unwindase, and other RNA-protein interactions (29, 30). DExD/H box proteins characteristically contain seven to eight conserved motifs including the

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The abbreviations used are: HCMV, human cytomegalovirus; OBP, origin-binding protein; HSV-1, herpes simplex virus type 1; EBV, Epstein-Barr virus; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; AC, arginine-conjugated; CIP, calf intestinal alkaline phosphatase; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; HHV8, human herpesvirus 8; T-ag, T-antigen.
Walker A and B motifs that are thought to be the sites responsible for binding and hydrolysis of NTPs (29–31). We now show for the first time that UL84 is a phosphoprotein that has UTPase activity displaying characteristics that are similar to other OBPs as well as members of the DEcDH box family of proteins. The UTPase activity of UL84 may be part of an energy-generating system for helicase activity involved in the initiation of HCMV DNA replication.

**EXPERIMENTAL PROCEDURES**

**Cells, Viruses, and Plasmids**—Vero cells were propagated and infected with Ad84 or transfected with pFLAG-control (Stratagene) as described previously (27).

**In Vivo Labeling and Immunoprecipitation**—Vero cells were seeded at 70% confluence in 10-cm dishes in DMEM supplemented with 10% fetal bovine serum (FBS). Cells were infected 24 h after seeding with Ad84 using a multiplicity of infection of 5. Cells were washed with PBS and lysed with 1 ml of lysis buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100) for 30 min at room temperature with shaking. The cell lysate was collected, and the sample was passed through a 22-gauge needle. The preparation was centrifuged at 3,200 × g for 5 min to clear the lysate of cell debris. The lysate was precleared using agarose-DEA beads as per the manufacturer’s protocol (Sigma). Before elution some samples were treated with calf intestinal alkaline phosphatase (CIP) (New England Biolabs) according to manufacturer’s recommendations. The beads were boiled in SDS sample buffer containing 15% β-mercaptoethanol and washed on a 10% SDS-PAGE gel. The gel was dried and analyzed by autoradiography or alternatively immunoblotted and probed with anti-FLAG antibody (Sigma).

**Phosphoamino Acid Mapping**—UL84 was in vivo labeled, harvested, and immunoprecipitated using the herein described method. Phosphoamino acid mapping was performed essentially as described previously (32) with the small difference that the gel was blotted on polyvinylidene difluoride membrane (Millipore) and visualized using India ink stain prior to hydrolysis of the excised band.

**UL84 Protein Purification**—UL84 protein was purified as described previously (27) via FLAG affinity chromatography. Alternatively, a reduced salt preparation of UL84 was purified from recombinant adenovirus (Ad84)-infected Vero cells 72 h post infection by scraping cells into ice-cold 50 mM Tris-HCl (pH 7.5) with protease inhibitors (Sigma) and lysing the cells using a nitrogen bomb (Kontes) under a pressure of 500 p.s.i. for 30 min. The lysate was subsequently treated as previously described (27). All protein samples were tested by Coomassie-stained SDS-PAGE. As a control we used mock adenovirus or AdΔTrack-infected (recombinant adenovirus expressing enhanced green fluorescent protein) Vero cell lysate for FLAG affinity purification. Only proteins that were >95% pure were used in NTPase assays.

**NTPase Assays**—NTPase activity was assayed by measuring the amount of product in the form of free phosphate (P) (ATP and GTP) or NDP (CTP and UTP) by quantifying the separation of radioactive products from the NTP substrate. The typical total reaction of 120 μl contained 50 mM HEPES (pH 7.5), 1 mM NTP (United Biochemical), 0.125 μCi (41.6 nM) each of [γ-32P]ATP (3,000 Ci/mmol), [γ-32P]GTP (3,000 Ci/mmol), [γ-32P]UTP (3,000 Ci/mmol), and [γ-32P]CTP (3,000 Ci/mmol) (Perkin Elmer Life Sciences), and 5–10 pM of UL84; this mixture was incubated for 20 min at 37 °C to prewarm the enzyme before adding MgCl2 to a final concentration of 6 mM to start the reaction. 10-μl aliquots of the reaction were removed and mixed with 10 μl of 0.25 mM EDTA at the appropriate times to stop the reaction (see the legends for Figs. 3 and 4). 2 μl of this mixture was spotted in 1-μl aliquots onto polyethyleneimine-cellulose TLC plates (EMD Chemicals Inc.) and after drying developed in 1 M formic acid and 0.4 M LiCl. The separated reaction products were visualized and quantified using the Personal Molecular Imager FX System (Bio-Rad).

**RESULTS**

**UL84 Is Phosphorylated at Serine Residues**—All known herpesvirus OBPs are phosphorylated by cellular kinases. To determine whether UL84 was phosphorylated by cellular kinases and assess the similarities between UL84 and other OBPs, we obtained purified UL84 from Vero cells infected with a recombinant adenovirus expressing UL84-FLAG fusion protein. Alternatively, Vero cells were transfected with a FLAG control plasmid that expressed the luciferase protein fused with FLAG as an unphosphorylated control protein. The proteins were in vivo labeled, harvested, and immunoprecipitated as described under “Experimental Procedures.” One sample of each protein was treated with CIP as a control to ensure that the phosphates could be removed from the proteins. Protein samples were resolved on a 10% SDS-PAGE gel. No band was detected in samples that contained control FLAG protein, whereas a radioactive band was detected that corresponded to UL84 (Fig. 1A, lanes 1 and 3, respectively). Samples were also treated with CIP to remove radiolabeled phosphates. The UL84 radioactive band was no longer detected in the sample treated with CIP (Fig. 1A, lane 4). Western blot analysis was performed using the same protein sample to show that CIP treatment did not appreciably affect protein concentrations (Fig. 1B).

To determine which amino acids were phosphorylated we performed phosphoamino acid mapping. UL84 protein was again in vivo labeled and immunoprecipitated. The resulting protein band was transferred to a PDVF membrane and hydrolyzed with hydrochloric acid. The released phosphoamino acids were separated by two dimensional Hunter thin layer electrophoresis along with unlabeled phosphoamino acid standards. The resulting TLC plate was visualized by autoradiography. The autoradiograph showed that UL84 is primarily phosphorylated at serine residues when compared with the phosphoamino acid standards (Fig. 1C). Fig. 1D is a depiction of all of the serine residues that are possible candidates for phosphorylation in the UL84 sequence as predicted by NetPhos 2.0. Fig. 1 displays strong evidence that UL84 is phosphorylated by cellular kinases at serine residues.

**UL84 Is a Putative Member of the DEcDH Family of Helicases**—UL84 exhibits only very limited homology to any functional group of proteins when examined by classical BLAST searches; however, the current data gathered concerning UL84 indicates that the protein is a functional homologue to UL9 and other herpesvirus OBPs. We choose to compare UL84 to DEcDH box family of protein, since many other viral initiation proteins fall into this category. Upon careful comparison to the DEcDH helicase motifs, it became clear that UL84 protein contained many of these motifs. Fig. 2 shows the alignment of UL84 with several DEcDH protein families including DEAH, DEAD, DEExH, SFN2, Rd3, and UL9. UL84 contains motifs homologous to a combination of the different DEcDH families. The Walker A motif of UL84 is slightly divergent from the other families shown in Fig. 2; however, it contains significant homology to Walker A motifs from bacteriophage proteins with the conserved arginine in the third position (33). The UL84 motif Ia is homologous to the DEAH family of RNA helicases and exhibits a high degree of identity with this family. The Walker B motif of UL84 is highly homologous to the UL9 Walker B motif as is displayed in the inset panel of Fig. 1 and also shows amino acids with the Walker B motifs of SV40 large T-antigen and papillomavirus E1-11. UL84 again exhibits homology to the DEAH family of helicases based on the auxiliary sequence LxxY that precedes the Walker B motif. UL84 also contains a motif IV homologous to the DEExH and Rd3 families of helicases that is flanked by auxiliary sequences and a motif V that are found in the DEAH family. UL84 does not appear to
contain an identifiable motif VI in its amino acid sequence. Taken together, these observations strongly suggest that UL84 is a member of the DE\(\times\)D/H box family of proteins and may exhibit any number of functions associated with this family of enzymes.

UL84 Displays UTPase Activity—Since we hypothesized that UL84 had enzymatic activity based on the motifs found in the sequence, we developed an in vitro assay to determine whether purified UL84 can hydrolyze NTPs (27). Many of the procedures used for UL84, including buffers and salt concentrations, were adapted from the well characterized HSV-1 UL9 protein, and this served as a starting point for the initial characterization the NTPase activity of UL84. Based on assays developed for the UL9 enzyme, we initially used ATP as a substrate for hydrolysis in our assay involving UL84. However, this nucleotide was poorly hydrolyzed in comparison with UTP. Consequently, we incubated purified UL84 with UTP and assayed for hydrolysis as described under “Experimental Procedures.” Our first step in the kinetic characterization of UL84 UTPase activity was the determination of the reaction order, which served to authenticate our kinetic data. When increasing concentrations of UL84 were added to the reaction mixture a linear rate of hydrolysis occurred indicating that the enzyme concentration was proportional to increased velocity of the reaction (Fig. 3A). This result strongly suggested that UL84 displayed kinetics consistent with a first order reaction and that kinetic data using this method was valid.

UL84 Nucleic Acid Stimulation and Enzymatic Stability—Since UL84 was shown to display first order kinetics, the next

![Fig. 1. UL84 is phosphorylated at serine residues. A, in vivo phosphorylation of anti-FLAG immunoprecipitated UL84. Lane 1, FLAG control protein; lane 2, FLAG control protein treated with CIP; lane 3, UL84; lane 4, UL84 treated with CIP. B, Western blot of anti-FLAG immunoprecipitated protein. Lane 1, FLAG control protein; lane 2, FLAG control protein treated with CIP; lane 3, UL84; lane 4, UL84 treated with CIP. C, phosphoamino acid analysis of UL84 by two-dimensional Hunter thin layer electrophoresis in buffers (pH 1.9 and pH 3.5). Non-radioactive phosphoamino acid controls were visualized with 0.25% ninhydrin, and their position is indicated in the figure. D, NetPhos 2.0 prediction of serine phosphorylation in UL84 sequence.](http://www.jbc.org/)

![Fig. 2. UL84 is a putative DE\(\times\)D box protein. The consensus sequence motifs of DE\(\times\)D/H families are presented, where \(\times\) indicates any amino acid. Darkly shaded areas represent identical amino acids shared between the corresponding families. Lightly shaded areas in the inset figure of motif II indicate analogous amino acid substitutions between the corresponding proteins.](http://www.jbc.org/)

![Motifs](http://www.jbc.org/)

NTP binding/hydrolysis Walker A Motif

Motif II

UL9

UL84

SV40

E1-1
step was to investigate the properties of UL84 UTP hydrolysis with respect to the ability to retain enzymatic function over time. DEH/DH family members often display nucleic acid dependence with respect to their NTPase activity. To evaluate UL84 nucleic acid dependence we performed our UTPase assay with the addition of ssRNA (poly(A)), dsDNA (activated calf thymus DNA), or ssDNA (poly(dT)). The addition of these nucleic acid substrates resulted in a slight enhancement of UTPase activity (Fig. 3B). This result indicated that perhaps the enzymatic activity was only slightly affected by the addition of nucleic acid using the present reaction buffer. Other explanations for only a modest increase in enzymatic activity from the addition of nucleic acids to the reaction mixture include the possibility that UL84 requires a sequence-specific substrate or that the proper substrate or reaction conditions have not yet been determined. Also, additional viral and/or cellular proteins may be required for an optimal nucleic acid interaction and subsequent UTP hydrolysis by UL84.

To show that UL84 was responsible for the hydrolysis reaction, we performed several control reactions. We heat-treated UL84 at 95 °C for 2 min prior to adding the protein to the reaction mixture. As a result, only minimal hydrolysis was observed, indicating that the denatured protein-cation-nucleotide complex was not sufficient to hydrolyze UTP (Fig. 3B). In addition, the omission of MgCl$_2$ or UL84 itself from the reaction mixture resulted in no measurable increase in UTP hydrolysis (Fig. 3B). Mock Vero cell lysate FLAG affinity column purification also exhibited a minimal amount of hydrolysis, which indicated that the UL84 protein was responsible for the UTPase activity (Fig. 3B). These results suggested that the UTPase activity of UL84 was stimulated by nucleic acid and indicated that activity was dependent upon the presence of the divalent cation Mg$^{2+}$ and was heat-sensitive. Also, the failure of mock-infected cell lysates to show any UTPase activity added a high degree of confidence that no contamination with cellular UTPases accounted for the observed activity.

Incubation of UL84 with UTP resulted in a linear increase in hydrolyzed product for up to a reaction time of 30 min (Fig. 3C).
Each cation was used at the same concentration in the standard reaction mixture. The thymus DNA and CTP, UTP, GTP, or ATP as substrates in the reaction (Fig. 3). Reactions were performed using activated calf degree (Fig. 3). NTPs, the enzyme was able to hydrolyze UTP to the highest regression (Prism 4.3). Although UL84 was able to utilize all NTPs, the enzyme was losing activity and no observed product inhibition at initial stages of the reaction. The velocity of the reaction slowed beyond 30 min, which may indicate that the enzyme was losing catalytic sites due to product inhibition or loss of protein conformational stability. Also shown is a TLC plate for a representative time course experiment displaying both substrate and hydrolysis product (UDP) (Fig. 3C, bottom panel).

**UL84 NTPase Kinetics**—Although our initial results indicated that UTP was efficiently hydrolyzed by UL84, we sought to further define the ability of the enzyme to utilize alternative NTPs as well as characterize UL84 enzyme kinetics. NTP hydrolysis was measured as described under “Experimental Procedures,” and the $V_{\text{max}}$ and $K_m$ values were calculated based on hyperbolic fit to the Michaelis-Menten equation by non-linear regression (Piasm 4.3). Although UL84 was able to utilize all NTPs, the enzyme was able to hydrolyze UTP to the highest degree (Fig. 3D). Reactions were performed using activated calf thymus DNA and CTP, UTP, GTP, or ATP as substrates in the reaction mixture. The $V_{\text{max}}$ value for UTP was ~6-fold higher than that observed for CTP (Fig. 3D). $V_{\text{max}}$ values for ATP and GTP were significantly lower (5.454 and 3.768 pmol/min, respectively) than that measured for UTP (Fig. 2D). The calculated $K_m$ values for UTP and CTP were similar (9.8 and 5.7 mM, respectively), whereas the $K_m$ values for ATP and GTP (1.1 and 1.9 mM, respectively). These data indicate that although UL84 can utilize all NTPs, UL84 displayed a preference for UTP as determined by the $V_{\text{max}}$ to $K_m$ ratio that is 6-fold higher for UTP than CTP (Fig. 3D).

**UL84 Enzymatic Characteristics**—We next investigated the buffer constituents and reaction conditions that contributed to the activity of UL84. First, we wanted to determine the optimal pH at which UL84 is most active. UTPase assays were performed using the standard buffer except various pH values were used. The UTPase activity of UL84 was determined at pH values ranging from 6 to 8.5 (Fig. 4A). Optimal activity was observed at a pH value of 7.5, and the optimal range was determined to be between pH 7 and 8 (Fig. 4A). We also investigated the effect of lowered or increased temperature on UL84 UTPase activity. We found that UL84 activity increased with respect to temperatures up to 45 °C (Fig. 4B).

The UTPase activity of UL84 using various concentrations of either NaCl or KCl was also examined. Independent of the salt used, we observed a decrease in activity at concentrations above 20 mM (Fig. 4C). This decrease continued and became more severe at NaCl concentrations of 250 mM (Fig. 4C). KCl has less of an effect on enzyme activity, in that UTP hydrolysis decreased only slightly even at KCl concentrations of 200 mM (Fig. 4C). These results suggested that higher concentrations of NaCl may disrupt some essential tertiary order structure of UL84. UL84 was also found to prefer $\text{Mg}^{2+}$ as the divalent cation required for the reaction. $\text{Mn}^{2+}$, $\text{Ca}^{2+}$, and $\text{Zn}^{2+}$ were also able to catalyze the reaction to a lesser degree, respectively (Fig. 4D). $\text{Mg}^{2+}$ is commonly the divalent cation of choice for DExD/H box proteins and most helicases.

**DISCUSSION**

Herpesvirus lytic DNA replication involves a cis-acting origin and a set of transacting factors. Lytic origins are quite diverse among the herpesviruses. For the prototypical alphaherpesvirus, HSV-1, the minimal lytic origin contains A-T rich regions and DNA elements that interact with the OBPs VP16 (34–38). The HSV-1 lytic origin and the mechanism of initiation are the best characterized of the herpesviruses. The lytic origins for the gammaherpesviruses, EBV and human herpesvirus 8 (HHV8), appear to be more complex than the HSV-1 lytic origin. These origins contain strong promoters/enhancers and initiate DNA synthesis possibly through a step that involves transcription or the formation of DNA primers (39–42). In addition to the presence of these strong promoters, HHV8 and EBV lytic origins contain distinct DNA elements that also interact with initiator proteins. These initiator proteins, Zta for EBV and ORF50 and K-bZIP for HHV8, interact with response elements that exist within the orILyt regions (42–46). The HCMV mechanism of orILyt recognition and initiation appears to have similarities to both the alphaherpesviruses, and the gammaherpesviruses, however, is distinct from both groups.

The most notable feature of the HCMV orILyt is a region that contains an RNA-DNA hybrid structure (47). This region is adjacent to a stretch of DNA that contains a strong UL84/E2-responsive promoter. Although the function of this RNA-DNA structure is unknown, it could act as a specific substrate for a UL84 putative helicase activity.

The first evidence that UL84 has an enzymatic function was presented here by our data. We propose that UL84 is a helicase based on a high degree of amino acid homology to RNA or DNA helicases/unwindases belonging to the DExD/H box family. Although helicases in this family are a diverse group, UL84 appears to have close identity to the DExD/H motifs of other DNA virus initiators proteins from HSV-1, polyoma, and papillomaviruses as is demonstrated by the homology of the Walker B motifs of UL84 to UL9, E1-11, and SV40 large T-antigen. Walker A and B sites are involved in NTP binding and hydrolysis (31) and are present in the majority of DNA helicases as well as many DNA synthesis initiator proteins. For UL84, amino acid analysis clearly indicates the presence of the consensus motifs for Walker A and B sites in addition to Ia, IV, and V. Interestingly, although UL84 has a high degree of homology to motifs Ia, II (Walker B), IV, and V, which are all found at the C-terminal portion of the protein, motif I (Walker A) of UL84 is less conserved and is found at the N terminus of the protein. UL84 does not contain motif VI, which was also implicated in NTP hydrolysis (48). These deviations may have implications for UL84 efficiency of catalysis or may indicate the...
HCMV Phosphoprotein UL84 Has UTPase Activity

need for a binding partner to supply additional sites of NTP hydrolysis for the enzyme to function efficiently.

Currently, there is no direct evidence that UL84 binds nucleic acid or exhibits strong nucleic acid dependence for enzymatic function; however, the protein does contain both the helicase motifs and an RGG box suggesting a preference for RNA or an RNA-DNA hybrid structure. UL9 exhibits strong stimulation with poly(dT), yet shows no stimulation with poly(dA) (2). This characteristic of UL9 indicates not only a preference for ssDNA but also for a specific ssDNA structure. Chromatin immunoprecipitation assays have shown that UL84 interacts with regions within oriLyt suggesting that other proteins (viral or cellular) may be involved.

SV40 large T-antigen (T-ag) was shown to have RNA unwinding activity, and this activity was powered by hydrolysis of non-A-T nucleosides such as UTP, which is also a characteristic of UL84 (18, 49). SV40 large T-antigen also shares epitopes with cellular RNA helicases and other DEAH-box proteins again similar to UL84 (49). In the case of SV40 T-antigen, this protein is active as a double hexamer. For papillomavirus E1 the double hexamer conformation is stabilized by E2 and these two proteins display helicase and NTPase activity (24). UL84 is also present as an oligomer in infected cells suggesting that the formation of a hexamer structure is possible.

DNA replication is a tightly regulated process and phosphorylation of OBPs is a mechanism by which DNA viruses control the initiation of DNA synthesis. In this respect, UL9, Zta, Or505, K-bZip, T-ag, and E1 proteins are all phosphoproteins. Phosphorylation is directly related to regulation of molecular interactions of Zta, T-ag, and E1 OBPs with their substrates (5, 16, 21, 22). Studies have also shown that UL9 ATPase activity is increased 5–8-fold upon phosphorylation by PKA (6). T-ag and E1 require the phosphorylation of specific amino acids to bind DNA, whereas Zta phosphorylation by CKII inhibits its interaction with DNA (16). Interestingly, the phosphorylation site required for DNA binding of E1 is in close proximity to a highly charged region of the protein. UL84 contains a highly charged region that is quite similar to this structure and contains potentially phosphorylated serine residues adjacent to this region. Although, at present we do not know if phosphorylation of UL84 contributes to activity, it is clear that the fact that UL84 is phosphorylated suggests that it may be a key factor in the regulation of viral DNA synthesis. The mechanism and biological affects of UL84 phosphorylation will be a subject of future study.

The $K_m$ of UL84 for hydrolysis of UTP is well within the established range for DEAH/helicases (50), especially dsRNA helicases; although, in comparison to UL9, the $K_m$ of UL84 is consistent with the unstimulated ATPase $K_m$ for UL9 (0.64 mM) (37). This may indicate that UL84 is more analogous to the RNA helicases. Other explanations for a minimal increase in activity upon the addition of nucleic acids include the possibility that we have not found the correct nucleic acid cofactor or that the protein is highly regulated by phosphorylation.

Most helicases, including DEAH proteins, are sensitive to high salt concentrations as was demonstrated with UL84. They also tend to function most efficiently at a pH between 7 and 8 or at a physiological pH, increase NTPase activity with respect to temperatures that are higher than physiological tempera-

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