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<td><strong>Author(s)</strong></td>
<td>Hew, Kelly; Veerappan, Saranya; Sim, Daniel; Cornvik, Tobias; Nordlund, Pär; Dahlroth, Sue-Li</td>
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Structure of the Open Reading Frame 49 Protein Encoded by Kaposi’s Sarcoma-Associated Herpesvirus

Kelly Hew,a Saranya Veerappan,a Daniel Sim,a Tobias Cornvik,a Pär Nordlund,a,b,c Sue-Li Dahlrotha
Division of Structural Biology and Biochemistry, Nanyang Technological University, School of Biological Sciences, Singaporea; Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Swedenb; Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singaporec

ABSTRACT Herpesviruses alternate between the latent and the lytic life cycle. Switching into the lytic life cycle is important for herpesviral replication and disease pathogenesis. Activation of a transcription factor replication and transcription activator (RTA) has been demonstrated to govern this switch in Kaposi’s sarcoma-associated herpesvirus (KSHV). The protein encoded by open reading frame 49 from KSHV (ORF49KSHV) has been shown to upregulate lytic replication in KSHV by enhancing the activities of the RTA. We have solved the crystal structure of the ORF49KSHV protein to a resolution of 2.4 Å. The ORF49KSHV protein has a novel fold consisting of 12 alpha-helices bundled into two pseudodomains. Most notably are distinct charged patches on the protein surface, which are possible protein-protein interaction sites. Homologs of the ORF49KSHV protein in the gammaherpesvirus subfamily have low sequence similarities. Conserved residues are mainly located in the hydrophobic regions, suggesting that they are more likely to play important structural roles than functional ones. Based on the identification and position of three sulfates binding to the positive areas, we performed some initial protein-DNA binding studies by analyzing the thermal stabilization of the protein in the presence of DNA. The ORF49KSHV protein is stabilized in a dose-responsive manner by double-stranded oligonucleotides, suggesting actual DNA interaction and binding. Biolayer interferometry studies also demonstrated that the ORF49KSHV protein binds these oligonucleotides.

IMPORTANCE Kaposi’s sarcoma-associated herpesvirus (KSHV) is a tumorigenic gammaherpesvirus that causes multiple cancers and lymphoproliferative diseases. The virus exists mainly in the quiescent latent life cycle, but when it is reactivated into the lytic life cycle, new viruses are produced and disease symptoms usually manifest. Several KSHV proteins play important roles in this reactivation, but their exact roles are still largely unknown. In this study, we report the crystal structure of the ORF49KSHV protein. This led to the discovery of novel DNA binding properties of the ORF49KSHV protein. Evolutionary conserved structural elements with functional homologs of ORF49KSHV were also established with the structure.

KEYWORDS Herpesvirus, Kaposi’s sarcoma-associated herpesvirus, KSHV, ORF49, structural biology
Kaposi’s sarcoma, primary effusion lymphoma, and multicentric Castleman’s disease (1–3).

All herpesviruses exist and propagate indefinitely in their hosts by having two distinct life cycles—the latent and the lytic life cycles (4). During the latent life cycle, the virus exists as a circular episome tethered to the host cell chromosome. The latent life cycle is characterized by limited expression of herpesvirus proteins, which are usually associated with maintenance of the viral episome and host immune evasion (5). Occasionally, the virus may be reactivated from the latent to the lytic life cycle, and disease symptoms usually surface. Even though the process of reactivation is not well defined, the overall determining factor is a shift in immune response brought about by immune suppression related to factors like organ transplants or AIDS, fever, stress, UV light exposures, and even menstruation (6–12). During the lytic life cycle, the full repertoire of herpesvirus proteins is expressed in a cascaded fashion, starting from the immediate early genes to the early genes and finally the late genes (13). New infectious virus particles are assembled and subsequently released upon host cell lysis (14).

KSHV encodes a protein called replication and transcription activator or RTA. RTA is a transcription factor that has been determined to be the molecular switch responsible for reactivation and is sufficient to drive the expression of all KSHV lytic genes (15–17). Another KSHV-encoded protein, the open reading frame 49 protein (ORF49KSHV), has been shown to upregulate the transcriptional activity of RTA (18). The protein homologs of ORF49KSHV in Epstein-Barr virus (EBV) and murine herpesvirus 68 (MHV-68) display similar effects in the respective viruses (19–22). ORF49KSHV has been demonstrated to enhance lytic replication in KSHV, while the lack of the ORF49MHV-68 protein has been shown to attenuate viral growth of MHV-68 (23). It is likely that the complementing effects of ORF49KSHV and its homologs on the lytic switch protein RTA are important for the lytic replication in the gammaherpesvirus subfamily.

To date, there is a lack of structural information on individual herpesviral proteins. To increase the knowledge of the ORF49KSHV protein and its homologs, we sought to characterize the protein by solving the crystal structure. Structural analysis of ORF49KSHV revealed a novel fold with sulfate ions binding to the distinctively charged protein surface. These data prompted DNA binding studies of the ORF49KSHV protein using a thermal stability shift assay (TSSA) and biolayer interferometry (BLI). Both TSSA and BLI were able to demonstrate nonspecific DNA binding by the ORF49KSHV protein with low micromolar affinities. DNA binding may contribute to the function of ORF49KSHV during lytic replication. By comparing the locations and interactions of conserved amino acid residues in all homologs and also analyzing the secondary-structure predictions, it is with confidence that we propose that all ORF49KSHV protein homologs have the same overall fold and structure. It, however, remains to be shown if all homologs display the same DNA binding properties as the KSHV protein.

RESULTS

Crystal structure of the ORF49KSHV protein. The full-length ORF49KSHV protein (amino acids 1 to 301) was cloned, expressed, purified, and crystallized. The structure was determined with the single anomalous dispersion (SAD) method and subsequently refined at a final resolution of 2.4 Å (Table 1). There is one ORF49KSHV molecule in each asymmetric unit, and each molecule comprises amino acids 10 to 300, with the exception of a disordered region from amino acids 135 to 143. Two point mutations (Q140P and Q179E) were identified in the amino acid sequence; both were verified by DNA sequencing and determined to have occurred during cloning.

The ORF49KSHV protein has an elongated structure made up of 12 alpha-helices (Fig. 1a). A structural comparison of the ORF49KSHV protein against protein databases showed that ORF49KSHV adopts a novel fold (24, 25). A long single helix, α3, spans the entire length of the structure and kinks in the middle, creating a slight “V” shape with a groove on one face of the protein surrounded by two pseudodomains. Pseudodomain 1 consists of the N-terminal part of α3 (residues 41 to 69) that creates a helical bundle with alpha-helices α1 and α2 and α10 to α12 (Fig. 1b, top). Pseudodomain 2
consists of the C-terminal part of α3 (residues 70 to 89) and helices α4 to α9 (Fig. 1b, bottom). The entire protein is stabilized by coiled-coil interactions, and in both pseudodomains, the majority of the helices are slightly twisted around each other (Fig. 1b). The two point mutations mentioned above are located within a disordered surface loop (Q140P) and the protein surface (Q179E), respectively, and do not seem to disrupt the structural integrity of the protein or to affect the structural analysis of the ORF49KSHV protein.

Three sulfate ions (S1 to S3) were successfully modeled into the positive spherical electron densities on the surface of each ORF49KSHV molecule (Fig. 1a). These sulfate ions are likely to have been contributed from the crystallizing solution. S1 is found near the C-terminal end of the protein and is directly coordinated by His 270 and Arg 273 on α11 (Fig. 1c). S2 is located near α6 and α7 and is coordinated by Arg 160 and Arg 164 (Fig. 1d). S2 also binds to the main chain amide of Gly 157 and the side chain of Ser 162 through a water-mediated interaction involving W19. S3 is located in a patch of histidine residues on α12 and is coordinated by His 293 and His 296 (Fig. 1e).

An electrostatic potential surface map of the protein surface reveals that ORF49KSHV has a clear charge separation. One face of the protein has three characteristically

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**TABLE 1 Data collection and refinement statistics**

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<td>RMSD angles (°)</td>
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aThe values in parentheses are for the highest-resolution shell (2.5 to 2.4 Å).

bRmerge = \[
\frac{\sum_{i=1}^{n}(|I_i(hkl)| - |I(hkl)|)}{\sum_{i=1}^{n}(|I(hkl)|)}\] × 100, where I_i is the ith intensity measurement of reflection hkl and I(hkl) is the mean intensity measurement of the symmetry-related or replicated reflections of the unique reflection hkl.

cFOM, figure of merit; DM, density modification.

dRwork = \[
\frac{\sum_{i=1}^{n}(F_{obs}(hkl) - F_{calc}(hkl))}{\sum_{i=1}^{n}F_{calc}(hkl)}\] × 100, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively.

eRfree is equivalent to Rfactor, but 5% of the measured reflections have been excluded from refinement and set aside for cross validation.
charged patches. On this face, there are two positively charged patches flanking a negatively charged area in the middle (Fig. 2, side view 1). The opposite face of the protein is also highly charged (Fig. 2, side view 2). One end of this face is largely hydrophobic, while the other end is predominantly negative. The groove created by the protein’s slight “V” shape is largely hydrophobic (Fig. 2, top view). Conversely, the underside of the groove is highly charged with patches of negatively and positively charged surfaces (Fig. 2, bottom view). Sulfate ions are found on the positively charged surfaces of the protein.

**DNA binding studies of the ORF49<sub>KSHV</sub> protein using a TSSA and BLI.** The DNA binding abilities of ORF49<sub>KSHV</sub> were explored with different oligonucleotides, including the KSHV promoter DNA sequences of viral interleukin-6 (vIL-6), kaposin, ORF57, and polyadenylated nuclear RNA (PAN), as well as a random 28-mer (dsO<sub>28</sub>) and a random 14-mer (dsO<sub>14</sub>) oligonucleotide, using a TSSA (26). All oligonucleotides induced increased thermal stability to the melting temperature ($T_m$) of the ORF49<sub>KSHV</sub> protein, suggesting DNA binding by the protein. A 100 mM concentration of vIL-6 increased the $T_m$ of the ORF49<sub>KSHV</sub> protein by 4.97 ($\pm 0.07^\circ$C (Fig. 3a). Similarly, kaposin, ORF57, PAN, dsO<sub>28</sub> and dsO<sub>14</sub> also increased the $T_m$ of the ORF49<sub>KSHV</sub> Protein by 3.14 ($\pm 0.04^\circ$C, 3.88 ($\pm 0.07^\circ$C, 3.50 ($\pm 0.03^\circ$C, 2.96 ($\pm 0.50^\circ$C, and 2.37 ($\pm 0.18^\circ$C, respectively (Fig. 3a). All six oligonucleotides also induced a dose-dependent increment in the $T_m$ of ORF49<sub>KSHV</sub> protein (Fig. 3b). There was no detectable thermal stabilization by the oligonucleotides on human isocitrate dehydrogenase (NADP) cytoplasmic protein (IDH1), a non-DNA-binding citric acid cycle protein with a protein isoelectric point similar to that of ORF49<sub>KSHV</sub> (Fig. 3a) (27).
DNA binding kinetics of the ORF49<sub>KSHV</sub> protein measured using biolayer interferometry (BLI) further verified its DNA binding abilities. The ORF49<sub>KSHV</sub> protein could bind the promoter regions of vIL-6, kaposin, ORF57, and PAN with a $K_d$ (dissociation constant) of 2.56 ($\pm 0.08$) $\mu$M, 5.48 ($\pm 0.34$) $\mu$M, 9.05 ($\pm 0.82$) $\mu$M, and 2.58 ($\pm 0.07$) $\mu$M, respectively (Fig. 4). However, the ORF49<sub>KSHV</sub> protein could also bind dsO28 with a $K_d$ of 5.35 ($\pm 0.82$) $\mu$M and dsO14 with a $K_d$ of 5.68 ($\pm 0.44$) $\mu$M (Fig. 4).

Crystallization screening of ORF49<sub>KSHV</sub> with oligonucleotides has been attempted but has so far been unsuccessful.

**Sequence conservation in the ORF49 protein family.** The KSHV- and MHV-68-encoded ORF49 as well as the EBV-encoded Na protein are positional and functional homologs with limited sequence similarities (Fig. 5a). In an attempt to identify the important potential functional regions, the amino acid sequences of ORF49<sub>KSHV</sub>, ORF49<sub>MHV-68</sub>, and Na protein were aligned. Identical and similar amino acids were mapped onto the ORF49<sub>KSHV</sub> protein structure. Conserved residues are localized in

![Electrostatic potential surface map of ORF49<sub>KSHV</sub> protein. Four different orientations of the protein show that the surface of ORF49<sub>KSHV</sub> protein is characteristically charged with large areas of positively charged (blue), negatively charged (red), and hydrophobic (white) surfaces. Sulfate ions (S1 to S3), shown as spheres, are found binding to the distinctively charged protein surfaces of ORF49<sub>KSHV</sub>.](http://jvi.asm.org/)

**FIG 2**

**FIG 3** DNA binding studies of ORF49<sub>KSHV</sub> using TSSA. (a) The melting temperature ($T_m$) of ORF49<sub>KSHV</sub> protein was increased by the KSHV promoter regions of vIL-6, ORF57, PAN, kaposin, and two unrelated oligonucleotides, dsO28 and dsO14, in the TSSA. However, this was not observed with the control protein IDH1, which is a non-DNA binding protein (27). (b) ORF49<sub>KSHV</sub> with various concentrations of oligonucleotides (vIL-6, ORF57, PAN, kaposin, dsO28, and dsO14) showed a dose-responsive increment in the $T_m$ ($\Delta T_m$) of ORF49<sub>KSHV</sub>. The stabilization effects on ORF49<sub>KSHV</sub> appear to be higher with vIL-6, followed by ORF57, PAN, Kaposin dsO28 and dsO14, but the degrees of stabilization are not significantly different.

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hydrophobic regions along the interacting surfaces of the alpha-helices and most likely play a primarily structural role (Fig. 5b).

**DISCUSSION**

Reactivation into the lytic life cycle is important for the production of new infectious herpesvirus particles in the host and hence the survival of the virus (14, 28). In KSHV, this is governed by the transcriptional control facilitated by RTA, and studies have demonstrated that ORF49KSHV and its homologs are able to cooperate with RTA to enhance its transcriptional activation (13, 16–18, 20–22). However, the underlying mechanism of this process is still unclear, with limited functional studies and lack of structural information.

The structure of the ORF49KSHV protein was determined to obtain a better understanding of its role in the herpesviral life cycle. The ORF49KSHV protein has an elongated novel fold, and its surface shows a clear charge separation, suggesting several sites for interactions with other proteins and/or charged molecules (Fig. 1 and 2). The structure of ORF49KSHV is not conserved as DNA binding domains of typical transcriptional activators/repressors (24, 25). However, the presence of sulfate ions on its positive surfaces prompted us to investigate the ORF49KSHV protein’s potential DNA binding properties with oligonucleotides harboring the KSHV promoter regions upregulated by RTA (Fig. 1 and 2) (26). DNA binding was first investigated with a TSSA, which we have previously used successfully to identify DNA binding for viral proteins (29, 30).

A typical dose-responsive increase in protein stability in the presence of different oligonucleotides suggests that the ORF49KSHV protein is able to bind dsDNA in vitro (Fig. 3). Even though the stabilizing effects seem to be slightly stronger with the KSHV promoter DNA sequences than the unrelated oligonucleotides, the difference in $T_m$ is too small to clearly indicate a preference for the promoter sequences (Fig. 3b). BLI also demonstrated similar binding affinities of the ORF49KSHV protein with all the oligonucleotides. Even so, these preliminary results suggest that the ORF49KSHV protein is able to bind DNA of various lengths and sequences.

ORF49KSHV has been shown to increase transcriptional activation in cooperation with RTA (18). Studies with its MHV-68 homolog, ORF49MHV-68, suggested that this cooperation is likely due to interactions between ORF49MHV-68 and RTA (21). Since...
ORF49 of KSHV has been demonstrated to bind DNA in vitro, it is tempting to propose that the upregulation of RTA’s transcriptional activity by ORF49 of KSHV might also involve direct DNA binding by ORF49. Obviously, in vitro studies do not necessarily imply that ORF49 will show its DNA binding properties in infected cells.

For future work, it would be interesting to investigate the potential phosphate binding sites and also the DNA binding capacity in the presence of RTA through experimental approaches.
targeted mutations. As already mentioned, the sequence conservation between the different homologs is very low, and the residues that are involved in the coordination of the sulfate ions are not conserved. Instead, conserved residues seem to play structural roles rather than functional ones. Even so, the protein structure of ORF49KSHV constitutes the first structure to be solved of any of the ORF49 gammaherpesvirus protein homologs. A comparison of the secondary-structure predictions for the Na protein (data not shown) and the location of conserved residues indicate that Na and ORF49MHV-68 have a fold and overall structure similar to those of ORF49KSHV.

In summary, the structure of ORF49KSHV has provided us with insights into the protein, revealing a novel protein fold and potential interaction areas. DNA binding studies of ORF49KSHV also indicate direct binding between the ORF49KSHV protein and dsDNA. The DNA binding characteristics might be a part of the protein’s functional role in the lytic replication of KSHV.

MATERIALS AND METHODS

Protein expression. ORF49KSHV was cloned as previously described into pNIC28-Bsa4 with an N-terminal histidine tag to enable affinity purification and immunoblotting (31, 32). The starter culture was induced with 0.5 mM isopropyl-\(\beta\)-D-1-thiogalactopyranoside (IPTG) overnight. The cells were harvested by centrifugation at 4,500 \(\times g\) for 10 min at 15°C the next day. The pellet was flash frozen with liquid nitrogen, ground, and stored at \(-20°C\). Unless otherwise stated, all reagents were from Sigma-Aldrich.

Protein purification. The cell pellets were resuspended in ice-cold lysis buffer (100 mM HEPES [pH 8.0], 500 mM NaCl, 10 mM imidazole [pH 8.0], and 10% glycerol) supplemented with 0.5 mM tris(2-carboxyethyl)phosphine (TCEP), 0.1 mg/ml lysozyme, 1 \(\mu\)g/ml protease inhibitor cocktail (EDTA free; Nacalai), and 125 U/ml benzonase (Merck Millipore) and sonicated. The lysates were clarified by centrifugation (4,500 \(\times g\) for 25 min at 4°C) and filtration through a 1.2- \(\mu\)m syringe filter. The filtrate was loaded onto a preequilibrated 1-ml HisTrap HP IMAC column (GE Healthcare; 20 mM HEPES [pH 7.5], 500 mM NaCl, 10 mM imidazole, 10% glycerol, 0.5 mM TCEP), and the column was washed with 20 ml of equilibration buffer and 15 ml of wash buffer (20 mM HEPES [pH 7.5], 500 mM NaCl, 25 mM imidazole, 10% glycerol, 0.5 mM TCEP). The protein was eluted with 5 ml elution buffer (20 mM HEPES [pH 7.5], 500 mM NaCl, 500 mM imidazole, 10% glycerol, 0.5 mM TCEP), and the eluate was loaded onto a preequilibrated 16/60 Superdex 75; GE Healthcare) directly. The eluted protein samples were collected in 2-ml fractions and analyzed on 4 to 12% NuPAGE Bis-Tris gels. The pure ORF49KSHV protein samples were combined, and 2 ml TCEP was added prior to protein concentration. The ORF49KSHV protein was concentrated to 6 mg/ml using a 10-kDa MWCO protein concentrator, before being flash frozen with liquid nitrogen and stored at \(-80°C\).

TSSA. A thermal stability shift assay (TSSA) of purified ORF49KSHV protein with different oligonucleotides was performed using a real-time PCR (RT-PCR) machine (Bio-Rad) with a 96-well PCR plate (Bio-Rad).

Lyophilized oligonucleotides (Integrated DNA Technologies) were solubilized with deionized distilled water. Double-stranded oligonucleotides were prepared by mixing equimolar amounts of the complementary oligonucleotides (for vIL6, 5'-AAATGGGTGCTAACCCTGTCAAAA and 5'-TTTTGGACAGGGTAGC CACCCATT; kaposin, 5'-GGAAATGGGTGCTAACCCCTACATA and 5'-TTATGAGGGTGTCACCCCAT TTCC; ORF57, 5'-AGGTTAACAAATGTCCACCAGGC and 5'-GCCCAGGGAAACATTGTGGTACT; PAN, 5'- AAAACCCGCCCCCCTGTTCCACCTTT and 5'-AAGATTGAACACAGGGGGGCGGTT; dsO14, 5'-GCCCAAGGC CCCGGACCCGAGGACTT and 5'-AAAGTCTCGGGGCACGCGGCGTTG; dsO28, 5'-TATGTAACAATAATGTTCCCACGGC and 5'-TATGTAGGGGTTAGCCACCCATT; dsO28, 5'-GCCCAAGGC CCCGGACCCGAGGACTT and 5'-AAAGTCTCGGGGCACGCGGCGTTG). The oligonucleotides were incubated at \(95°C\) for 2 min and cooled at room temperature. Each well contained 0.2 mg/ml of the ORF49KSHV protein, 5 \(\times\) SYPRO orange (Life Technologies), and 100 \(\mu\)M oligonucleotides, marked up to a final volume of 25 \(\mu\)l with TSSA buffer (20 mM HEPES [pH 7.5] and 300 mM NaCl). Triplicate experiments were done, and TSSA buffer was added in place of the oligonucleotides as blanks. The same experiment was repeated by replacing the ORF49KSHV protein with purified human isocitrate dehydrogenase (NADP) cytoplasmic protein (IDH1) as a control. A dose-responsive TSSA of the ORF49KSHV protein and the oligonucleotides was also performed similarly to that described above, but without different oligonucleotide concentrations: 100 \(\mu\)M, 50 \(\mu\)M, 25 \(\mu\)M, 12.5 \(\mu\)M, 6.3 \(\mu\)M, 3.2 \(\mu\)M, 1.6 \(\mu\)M, and 0 \(\mu\)M.

BLI. The DNA-binding kinetics of the ORF49KSHV protein with various oligonucleotides were characterized with an Octet RED96 (Fortebio) biolayer interferometry (BLI) system at 25°C in the assay buffer (10 mM phosphate [pH 7.4], 137 mM NaCl, 2.7 mM KCl, 0.1% bovine serum albumin

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Cloning of full-length ORF49KSHV clone and Chen Dan (Nanyang Technological University, Singapore) for providing the recombinant purified IDH1 protein.

Portions of this research were undertaken on the MX1 and MX2 beam lines at the Australian Synchrotron, Victoria, Australia, and the 13B1 and 13C1 beam lines at the Australian Synchrotron, Australia, for providing the recombinant purified IDH1 protein.

Structure of ORF49KSHV was determined using the single-wavelength anomalous dispersion (SAD) method with the anomalous data set. SHELX-C and D were used for substructure determination, heavy-atom positional refinements, and phase calculations (36). The phases were further improved by density modifications with SHELX-E (36). An initial ORF49KSHV model was built automatically by ARP/wARP (37).

The native ORF49KSHV structure was determined using the single-wavelength anomalous dispersion (SAD) method with the anomalous data set. SHELX-C and D were used for substructure determination, heavy-atom positional refinements, and phase calculations (36). The phases were further improved by density modifications with SHELX-E (36). An initial ORF49KSHV model was built automatically by ARP/wARP (37).

The native ORF49KSHV structure was determined using the single-wavelength anomalous dispersion (SAD) method with the anomalous data set. SHELX-C and D were used for substructure determination, heavy-atom positional refinements, and phase calculations (36). The phases were further improved by density modifications with SHELX-E (36). An initial ORF49KSHV model was built automatically by ARP/wARP (37).

The final model was obtained after multiple cycles of automated refinements with phenix.refine and manual refinements with Coot (39, 40). The quality of the final model was validated using the comprehensive validation software from the PHENIX suite (43, 44). PDB2PQR and APBS from the APBS plug-in of the CCP4 suite were used for preparation of the protein for the calculation of the electrostatic potential.

Accession numbers. The final coordinates of ORF49KSHV were deposited in the PDB under structure ID 5IPX.

ACKNOWLEDGMENTS

We thank Jürgen Haas (University of Edinburgh, United Kingdom) for providing the full-length clone of ORF49KSHV, and Chen Dan (Nanyang Technological University, Singapore) for providing the recombinant purified IDH1 protein.

Portions of this research were undertaken on the MX1 and MX2 beam lines at the Australian Synchrotron, Victoria, Australia, and the 13B1 and 13C1 beam lines at the National Synchrotron Radiation Research Center, Taiwan.

REFERENCES


Chen VB, Arendall WB, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC. 2010. MolProbity:


47. DeLano W. 2014. The PyMOL molecular graphics system, version 1.7.2.0. Schrodinger, LLC.