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An Autophosphorylating but Not Transphosphorylating Activity Is Associated with the Unique N Terminus of the Herpes Simplex Virus Type 1 Ribonucleotide Reductase Large Subunit

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We report on a protein kinase function encoded by the unique N terminus of the herpes simplex virus type 1 (HSV-1) ribonucleotide reductase large subunit (R1). R1 expressed in *Escherichia coli* exhibited autophosphorylation activity in a reaction which depended on the presence of the unique N terminus. When the N terminus was separately expressed in *E. coli* and partially purified, a similar autophosphorylation reaction was observed. Importantly, transphosphorylation of histones and of proteins in HSV-1-infected cell extracts was also observed with purified R1 and with truncated R1 mutants in which most of the N terminus was deleted. Ion-exchange chromatography was used to separate the autophosphorylating activity of the N terminus from the transphosphorylating activity of an *E. coli* contaminant protein kinase. We propose a putative function for this activity of the HSV-1 R1 N terminus during the immediate-early phase of virus replication.

Ribonucleotide reductase (EC 1.17.4.1) catalyzes a rate-limiting step in the de novo synthesis of DNA, converting ribonucleoside diphosphates to the corresponding deoxyribonucleoside diphosphates (37). Herpes simplex virus type 1 (HSV-1) and HSV-2 both encode ribonucleotide reductase (7, 8, 11, 14, 30, 36) comprising a large subunit (R1; molecular mass, 136 kDa) and a small subunit (R2; molecular mass, 38 kDa), present in an $\alpha_2\beta_2$ heterodimer (2, 10, 14, 18). The R1 subunit contains the nucleotide-binding site and redox-active thiols, whereas the R2 subunit has two binuclear iron centers and provides the tyrosyl free radical involved in ribonucleoside diphosphate reduction (28, 33, 34). Amino acid sequence analysis indicates distinct homologies between HSV ribonucleotide reductase and the ribonucleotide reductases encoded by Epstein-Barr virus, varicella-zoster virus, vaccinia virus, *Escherichia coli*, bacteriophage T4, and mouse DNA (13). However, a feature unique to the R1 subunits of HSV-1 and HSV-2 is an N-terminal extension of some 320 amino acids which is absent from the R1 subunits of other species (27); for HSV-1, this N-terminal extension is unnecessary for ribonucleotide reduction in vitro (9, 13, 21).

HSV-1 and HSV-2 possess a protein kinase activity located within the unique N terminus of R1 (1, 3, 4, 29). In these studies, HSV R1 proteins, immunoprecipitated either from infected-cell extracts or from cells transfected with constitutive expression vectors, were observed to auto- and transphosphorylate. Expression of the N terminus of HSV-2 R1 in *E. coli* followed by its immunoprecipitation as a 29-kDa protein yielded, on analysis, a protein kinase activity (23).

Here we locate and characterize the phosphorylating activity of the HSV-1 R1 N terminus with (i) purified R1 expressed in *E. coli*, here termed EcR1, (ii) the N-terminal amino acids of R1 expressed separately in *E. coli*, and (iii) N-terminal truncations of R1 as reagents. HSV-1 R1 expressed in *E. coli* (EcR1) was purified as previously described (15). Plasmid pAET, encoding the truncated R1 mutant dN245R1, was prepared by deletion of the nucleotides encoding R1 amino acids 1 to 245 from the EcR1-expressing clone pETR1 type 1 (15) with exonuclease III and mung bean nuclease (Fig. 1). The remaining R1 portion was then inserted between the promoter and terminator of the expression vector pAET8C, a modification of pET8C (35) in which the backbone of the vector (pBR322) has been replaced by pAT153. Full details of this construct and the library of R1 truncations from which it was selected will be given elsewhere (14a). The expressed protein, termed dN245R1, was purified from *E. coli* extracts as described for intact EcR1 (15).

The first 449 amino acids of R1 were expressed in *E. coli* BL21 cells by using a modified pET vector (Fig. 1). The truncation was made by cutting the full-length R1 clone pYN-R1 (27) with *Bst*XI, and the end was rendered blunt with mung bean exonuclease. A new *Hin*dIII site was created by addition of linkers to the blunt *Bst*XI site, allowing the truncated R1 open reading frame to be removed on an *Xba*I-*Hin*dIII fragment for cloning into pET8C (the *Bam*HI site was filled in and *Hin*dIII linkers were added). The construct was confirmed by sequencing by the double-stranded dideoxy termination method (17, 32). pETdC449R1 was induced as described previously for full-length EcR1 (15).

The truncations dN246R1 and dN305R1 were prepared by proteolysis of purified EcR1 with chymotrypsin and trypsin, respectively (9); both proteases cleave at specific sites within the N terminus, chymotrypsin at amino acid 246 and trypsin at amino acid 305 (Fig. 1b).

Location and characterization of R1 kinase activity. The HSV-1 EcR1 kinase assays were performed with 300 ng of purified EcR1 in 20 μl of 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–250 mM NaCl–0.5 mM $MnCl_2$–0.1% (vol/vol) Nonidet P-40–2 μg of protamine per ml containing 1 μl of [γ-^{32}P]ATP (Amersham; specific activity, 5,000 mCi/mmol). The assay conditions were optimized as described below. Incubation was done at 25°C for 30 min, and reactions were stopped by adding 10 μl

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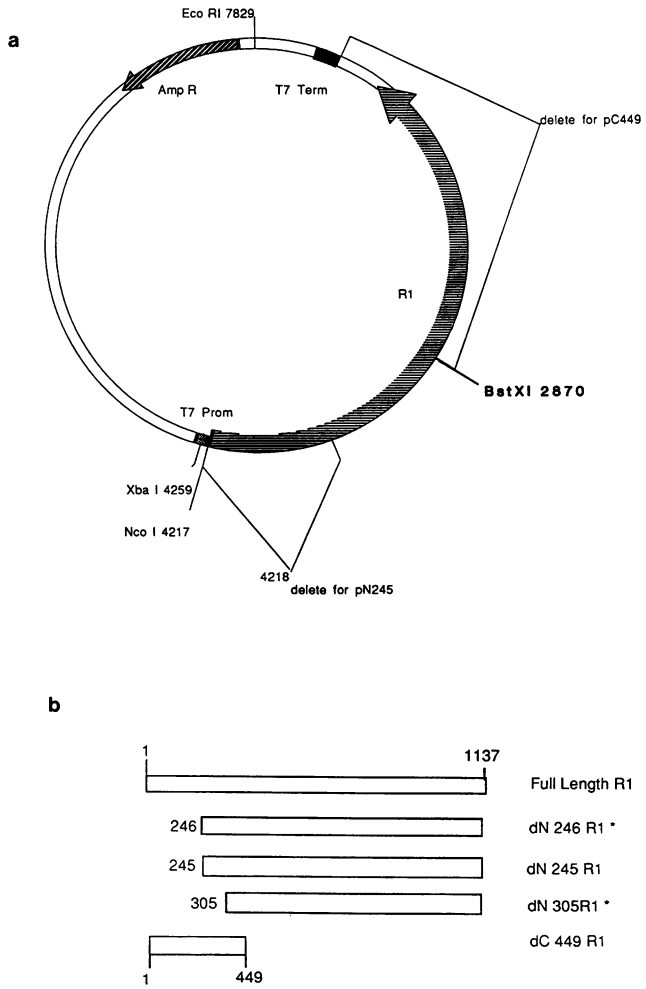

FIG. 1. (a) Map of R1 expression plasmid pETR1 type 1, showing regions deleted to produce pAETdN245R1 (pN245) and pETdC449R1 (pC449) as described in the text. Term, terminator; Prom, promoter. (b) Diagram of R1 polypeptides used in this study. Truncations marked with an asterisk were produced from full-length R1 by proteolysis. In the case of dN305R1, although a 30-kDa N-terminal fragment results from the tryptic cleavage, we do not know the location of this fragment within the N terminus (9), and hence it is not shown.

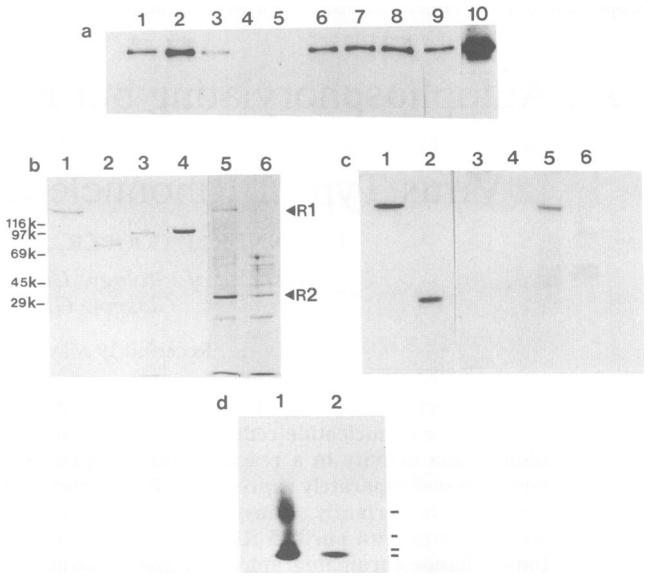

FIG. 2. (a) Determination of optimum conditions for EcR1 autophosphorylation by SDS-PAGE and autoradiography. EcR1 (300 ng) was incubated with 0, 250, 500, or 1,000 mM NaCl (lanes 1 to 4, respectively), with 0, 0.5, 1.0, or 2.0 mM Mn^{2+} (lanes 5 to 8, respectively), with no protamine (lane 9), and with 0.1 mg of protamine per ml (lane 10). Lanes 1 to 4 had 0.5 mM Mn^{2+}, lanes 5 to 8 had 250 mM NaCl, and lanes 9 and 10 had 0.5 mM Mn^{2+} and 250 mM NaCl. (b) Coomassie-stained SDS-PAGE gel showing (lane 1) purified recombinant EcR1, (lane 2) trypsin-treated EcR1 (dN305R1), (lane 3) chymotrypsin-treated EcR1 (dN246R1), (lane 4) purified dN245R1, (lane 5) R1 isolated from infected-cell extracts with an R2 affinity matrix, and (lane 6) mock-infected-cell extract incubated with the R2 affinity matrix. The positions of R1 and of R2 from the affinity matrix are indicated on the right-hand side of the gel. Molecular mass (in kilodaltons) is given on the left-hand side of the gel. (c) Analysis by SDS-PAGE and autoradiography of the proteins shown in panel b after kinase assays. Each protein (300 ng) was assayed under optimum conditions except in lanes 5 and 6, for which assays were performed in the presence of the R2 affinity matrix, as described in the text. Lanes are the same as in panel b. (d) Peptide map of (lane 1) autophosphorylated R1 isolated from infected-cell extracts and (lane 2) recombinant R1. V8 protease was used at 1 μg per well; after electrophoresis into the stacking gel, the power was turned off and the gel was incubated at 22°C for 1.5 h before resumption of electrophoresis. The gel was a 12.5% acrylamide gel, and the positions of marker proteins (Amersham) of 2.35, 3.4, 6.5, and 14.3 kDa are given on the right-hand side.

of 3× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (20). Incorporation of ^{32}P was determined by autoradiography of SDS-polyacrylamide gels. Bands corresponding to R1 were excised from the gel, and ^{32}P incorporation was quantified by liquid scintillation counting.

Figure 2a shows optimization of NaCl and Mn^{2+} concentrations for EcR1 autophosphorylation. Optimum activity occurred at 250 mM NaCl (Fig. 2a, lanes 1 to 4), and the reaction had an absolute requirement for Mn^{2+} (Fig. 2a, lanes 5 and 6), although concentrations of Mn^{2+} above 0.5 mM did not affect autophosphorylation (Fig. 2a, lanes 6 to 8). The basic protein protamine gave an approximately 10-fold stimulation of EcR1 activity, as shown in Fig. 2a, lanes 9 and 10.

Figure 2b shows a Coomassie-stained gel of purified EcR1 (lane 1), the proteolytic truncations dN305R1 and dN246R1 (lanes 2 and 3, respectively), purified dN245R1 (lane 4), and R1 obtained from the 45% $(NH_4)_2SO_4$ fraction of infected-cell extracts (lane 5) isolated with an HSV-1 R2 affinity matrix as described before (9); lane 6 represents the result of incubating the R2 affinity matrix with the equivalent fraction from mock-infected cells. The 45% $(NH_4)_2SO_4$ fractions from either HSV-1 strain 17^+-infected or mock-infected baby hamster kidney C13 cells were prepared as described by Dutia et al. (12). Figure 2c displays an autoradiograph of the proteins shown in Fig. 2b after the kinase assay. Material bound to the R2 affinity matrix (50 μl) was assayed by incubation with an equal volume of kinase assay buffer and 1 μl of [γ-^{32}P]ATP at 25°C for 30 min. After incubation, the matrix was washed twice in 25 mM HEPES buffer (pH 7.6) with 0.05% Nonidet P-40, and bound proteins were eluted with 25 μl of SDS-PAGE sample buffer.

EcR1 and R1 isolated from infected-cell extracts exhibited

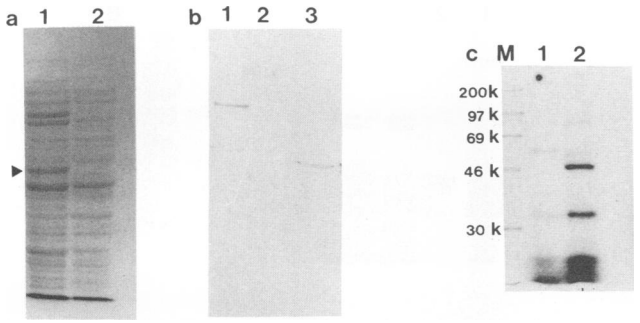

FIG. 3. Expression of amino acids 1 to 449 of HSV-1 R1 in *E. coli*. (a) Coomassie-stained gel showing (lane 1) induced and (lane 2) uninduced whole-cell extracts. The arrowhead indicates the novel polypeptide. (b) Western blot with monoclonal antibody 1026 of (lane 1) purified EcR1, (lane 2) uninduced whole-cell extracts, and (lane 3) induced whole-cell extracts. (c) Autoradiograph of 45% $(NH_4)_2SO_4$-precipitated fractions from (lane 1) uninduced and (lane 2) induced extracts after the kinase assay. Lane M, ^{14}C-labeled molecular mass markers (in kilodaltons).

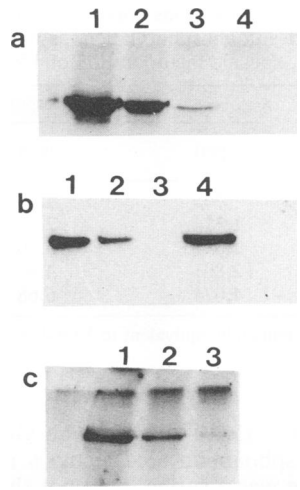

FIG. 4. Autoradiographs showing inhibition of EcR1 autophosphorylation by (a) unlabeled GTP at 0, 1, 10, and 100 μM (lanes 1 to 4, respectively); (b) AMP-PNP at 1, 10, 100, and 0 μM (lanes 1 to 4, respectively); and (c) FSBA at 0, 100, and 1,000 μM (lanes 1 to 3, respectively). In all cases, 300 ng of EcR1 was used under optimum conditions, as described in the text.

autophosphorylation (Fig. 2c, lanes 1 and 5). Treatment of intact EcR1 with trypsin generated a 30-kDa N-terminal fragment (9) which autophosphorylated (Fig. 2c, lane 2). This 30-kDa N-terminal fragment stains very poorly with Coomassie blue (9) and cannot be seen in Fig. 2b; however, it was detected by Western immunoblotting in this preparation. No phosphorylating activity was observed with the N-terminal truncations dN246R1 and dN245R1 (Fig. 2c, lanes 3 and 4) or the R2 affinity matrix incubated with mock-infected-cell extract (Fig. 2c, lane 6). Peptide mapping (6) was performed on autophosphorylated EcR1 or R1 from infected-cell extracts, and the results indicated that identical peptides were phosphorylated (Fig. 2d).

The induction by isopropylthiodigalactoside of a novel 46-kDa protein in whole-cell extracts of *E. coli* transfected with the plasmid pETdC449R1 is shown in Fig. 3a. This novel protein reacted with the HSV-1 R1-specific monoclonal antibody 1026 (24), the epitope for which maps within the N-terminal domain, on Western blotting (Fig. 3b). The additional faint bands occurring with EcR1 and the R1 N terminus are the result of degradation and were not observed consistently. Optimum precipitation of this 46-kDa protein was achieved with 45% $(NH_4)_2SO_4$, and when this fraction was assayed under EcR1 kinase assay conditions, the protein was phosphorylated (Fig. 3c); the other phosphorylated bands are *E. coli* proteins and were consistently observed in both induced and uninduced cell extracts. The 46-kDa phosphorylated R1 protein reacted with the monoclonal antibody 1026 in Western blots, and only this protein had requirements similar to those of intact R1 for Mn^{2+} and NaCl (data not shown). These data suggest that the autophosphorylating activity resides entirely within the N-terminal 449 amino acids of R1.

Substrate specificity and inhibitors. When EcR1 autophosphorylation was assayed in the presence of unlabeled ATP or GTP, incorporation of ^{32}P was inhibited. Figure 4a shows the effects of GTP at 0 μM (lane 1), 1 μM (lane 2), 10 μM (lane 3), and 100 μM (lane 4) on EcR1 autophosphorylation. At 100 μM, GTP inhibited activity by 90%, and almost identical inhibition was obtained with unlabeled ATP (data not shown), indicating that GTP can inhibit EcR1 autophosphorylation, probably by competing for ATP binding. The nonhydrolyzable ATP analogs 5′-adenylylimidodiphosphate (AMP-PNP) and 5′-fluorosulfonylbenzoyladenosine (FSBA) both inhibited EcR1 autophosphorylation. Inhibition by AMP-PNP was similar to that by unlabeled ATP or GTP (Fig. 4b), suggesting that this analog competes with the nucleotides for binding. FSBA was used at final concentrations of 1 mM and 100 μM, and because FSBA was dissolved in dimethylformamide, EcR1 activity was assayed with an equal volume of dimethylformamide as a control. Figure 4c shows an autoradiograph of EcR1 incubated with 1 mM (lane 3) or 100 μM (lane 2) FSBA or incubated with dimethylformamide (lane 1). ^{32}P incorporation was inhibited by 90% with 1 mM FSBA and by 50% with 100 μM FSBA. FSBA inactivates known protein kinases by reacting with an essential lysine residue within the nucleotide-binding site (16), and [^{14}C]FSBA labels protein kinases at this residue (19, 31). Although we were able to demonstrate labeling of EcR1 with [^{14}C]FSBA (NEN Du Pont), this labeling occurred outside the unique N-terminal region and was not inhibited by either 5 mM ATP or 5 mM GTP (8a). We note that the BCR gene in humans encodes a novel protein kinase which, although inhibited by FSBA, does not affinity label with this reagent (24).

In a further series of experiments, several kinetic parameters of EcR1 autophosphorylation were analyzed. Incorporation of ^{32}P was linear over a 20-min period, with no more incorporation after 60 min (data not shown). Incubation of various concentrations of EcR1 (from 75 to 600 ng) under kinase assay conditions for 10 min showed a linear relationship between EcR1 concentrations and ^{32}P incorporation (Table 1). From these data, determination of the rate of ^{32}P incorporation per milligram of protein (Table 1) indicated that the rate of EcR1 autophosphorylation was constant over this protein concentration range. A similar result was obtained when this experiment was repeated in the presence of 10 μM unlabeled ATP. These data demonstrate that R1 autophosphorylation is an intramolecular reaction.

The molar ratio of EcR1 to P_i was calculated to be 0.9. Calculation of this ratio from the results obtained in the presence of 10 μM unlabeled ATP gave a similar value (0.8).

TABLE 1. Effects of various concentrations of HSV-1 EcR1 on EcR1 autophosphorylation, as measured by specific incorporation of ^{32}P[a]

R1 concn (ng/assay)	^{32}P incorporated		
	(cpm)	fmol	pmol/μg of R1
75	702	1.17	15.6
150	1,314	2.19	14.6
300	2,041	3.40	11.3
450	2,801	4.67	10.4
600	4,074	6.68	11.1

[a] 600 cpm is approximately equivalent to 1 fmol of ^{32}P.

We conclude that EcR1 autophosphorylation occurs at a single site; phosphoamino acid analysis indicated that the modified residue was a serine (data not shown).

Transphosphorylating activity. The ability of EcR1 to phosphorylate exogenous substrates (at 0.1 mg/ml) was investigated with histones, protamine, casein, calmodulin, and proteins present in heat-inactivated (60°C, 1 h) extracts from HSV-1-infected cells. No transphosphorylation reactions were observed when EcR1 was incubated under kinase assay conditions with protamine, casein, or calmodulin (data not shown). By contrast, transphosphorylation reactions were observed when EcR1 or trypsin-treated EcR1 was incubated with calf thymus histones or heat-inactivated proteins from infected-cell extracts (Fig. 5a, lanes 1, 2, and 7). The additional band in lane 2 is the 30-kDa N-terminal fragment produced by trypsin treatment of EcR1, which retains autophosphorylating activity. When histones or the heat-inactivated proteins were incubated under identical conditions but without EcR1, no phosphoprotein bands were observed (Fig. 5a, lanes 5 and 6).

Significantly, when the two N-terminal deletions dN245R1 and dN246R1 were incubated with histones, phosphorylated proteins identical to those observed with EcR1 were seen (Fig. 5a, lanes 3 and 4). Phosphorylation also occurred when dN245R1 was incubated with heat-inactivated infected-cell proteins (Fig. 5a, lane 8). As these truncations have most of the R1 N terminus deleted, we conclude that the transphosphorylating activity is not associated with the unique N terminus but is the result of a contaminant *E. coli* protein kinase. This was confirmed by fast protein liquid chromatography (FPLC) on a Mono Q ion-exchange column, which separated EcR1 autophosphorylation from histone transphosphorylation (Fig. 5b). This was achieved by applying purified EcR1 to a Mono Q column and eluting bound protein with a 0 to 0.5 M NaCl gradient developed over 15 ml. Every third fraction of this gradient was desalted with an FPLC fast-desalt column and assayed for EcR1 autophosphorylation and histone transphosphorylation. EcR1 autophosphorylation was observed in fraction 15, whereas histone phosphorylation occurred in fraction 9.

These studies demonstrate the presence of an autophosphorylating activity associated with the unique N terminus of HSV-1 R1. This activity resembles protein kinase activities in that it is inhibited by the ATP analogs AMP-PNP and FSBA. Despite using a variety of known substrates for other protein kinases, we were unable to observe any transphosphorylation activity. Our results suggest that the activity was restricted to autophosphorylation.

General discussion. Chung et al. (4) have identified amino acid sequence homologies between the N termini of HSV-2

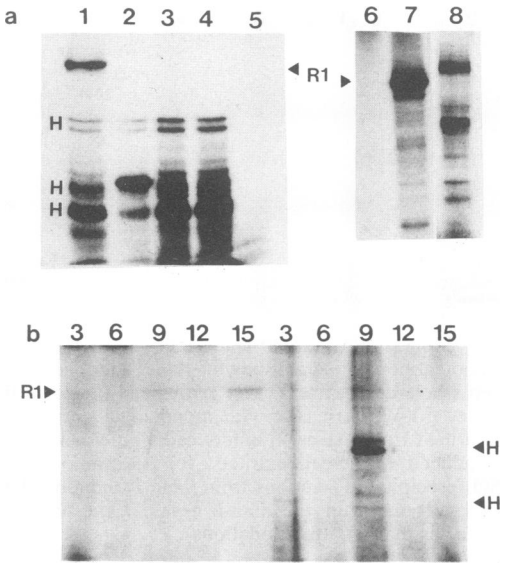

FIG. 5. (a) Transphosphorylation of substrates by EcR1 and R1 N-terminal deletions. Assays were performed with 300 ng of R1 protein under optimum conditions in the presence of 0.1 mg of exogenous substrate per ml. Lanes 1 to 5, histones with (lane 1) EcR1, (lane 2) dN305R1, (lane 3) dN246R1, lane 4) dN245R1, and (lane 5) no R1 (kinase assay buffer only); lanes 6 to 8, heat-inactivated proteins from HSV-1-infected-cell extracts with (lane 6) kinase assay buffer, (lane 7) EcR1, and (lane 8) dN245R1. Histone positions are marked with H. (b) Separation of EcR1 autophosphorylation from histone transphosphorylation by FPLC Mono Q chromatography. Assays were performed on every third fraction, prepared as described in the text. The amount of protein in each fraction was not quantitated. However, examination of the eluted fractions by SDS-PAGE and Coomassie blue staining indicated optimal elution of R1 in fraction 15.

R1 and protein kinases and have shown that R1 autophosphorylates. Other studies indicated that HSV-2 R1 had the ability to transphosphorylate both histones and calmodulin and that the kinase domain had properties in common with growth factor receptor kinases, such as myristylation, membrane association, stimulation by polylysine, and a putative single transmembrane domain (3). Expression of amino acids 1 to 445 of HSV-2 R1 in *E. coli* and immunoprecipitation with specific antibodies identified a 29-kDa protein which exhibited autophosphorylation and transphosphorylated calmodulin (22). Ali et al. (1) have also reported autophosphorylation with R1 of HSV-1 and HSV-2 obtained by immunoprecipitation from infected-cell extracts. Paradis et al. (29) reported a protein kinase function associated with HSV-1 R1 from infected cells which showed both autophosphorylation and transphosphorylation of caesin and histones.

Our results confirm the ability of HSV-1 R1 to autophosphorylate. However, caution is necessary in interpreting R1 transphosphorylation results because we show that this activity in apparently homogeneous preparations of EcR1 was attributable to contamination.

Nevertheless, the N terminus of HSV-1 R1 could function as a protein kinase, as only a limited number of substrates were tested. If this region of R1 does function as a true protein kinase, the reaction mechanism is likely different from that of known protein kinases, as sequence homologies are very poor (16); a lysine residue essential for the activity of known protein kinases is absent.

Our results do not exclude the formal possibility that the kinase activity associated with the N terminus of HSV-1 R1 differs from that of the N terminus of HSV-2 R1, since the amino acid sequences of these regions show only limited homology (27) and there is a candidate lysine residue in the HSV-2 R1 N terminus (4). Paradis et al. (29), in their explanation of HSV-1 R1 kinase activity, speculated that the HSV-1 DNA sequence was incorrect and should contain a lysine equivalent to that in the HSV-2 R1 sequence, at amino acid 180. Two independent HSV-1 R1 clones have been sequenced which predict no lysine (25, 27), and the sequence of our clone, which expresses the 46-kDa N-terminal portion, agrees with these previously determined sequences (data not shown). Recently, Luo and Aurelian (22) have shown that the candidate lysine in HSV-2 R1 is not required for activity, which suggests that there may be some similarity in the reaction mechanisms of the two proteins.

During the restricted immediate-early phase of virus replication, the HSV-1 R1 polypeptide is present together with five other virus proteins which act to regulate expression of virus early and late genes (5, 38). The R1 promoter, which contains virus immediate-early motifs, is regulated as an immediate-early function (40), in contrast to the R2 gene, which behaves as a typical early function. This suggests a role for R1 during immediate-early infection which is distinct from ribonucleotide reductase activity. The phosphorylated R1 N terminus could function as a protein kinase during the immediate-early phase and phosphorylate at restricted sites on virus immediate-early or cellular proteins, thereby affecting transcriptional activity. In this regard, the hepatitis B virus-encoded Hbx protein exhibits autophosphorylation and acts as a transcriptional activator; transactivation is achieved by a serine/threonine kinase activity which shows no amino acid homology to "conventional" kinases (39).

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REFERENCES

1. **Ali, M. A., D. McWeeney, A. Molosavljevic, J. Jurka, and R. J. Jariwalla.** 1991. Enhanced malignant transformation induced by expression of a distinct protein domain of ribonucleotide reductase large subunit from herpes simplex virus type 2. Proc. Natl. Acad. Sci. USA **88:**8257–8261.
2. **Bacchetti, S., M. J. Evelegh, and B. Muirhead.** 1986. Identification and separation of the two subunits of herpes simplex virus ribonucleotide reductase. J. Virol. **57:**1171–1181.
3. **Chung, T. D., J. P. Wymer, M. Kulka, C. C. Smith, and L. Aurelian.** 1990. Myristylation and polylysine-mediated activation of the protein kinase domain of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10). Virology **179:**168–178.
4. **Chung, T. D., J. P. Wymer, C. C. Smith, M. Kulka, and L. Aurelian.** 1989. Protein kinase activity associated with the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10). J. Virol. **63:**3389–3398.
5. **Clements, J. B., R. J. Watson, and N. M. Wilkie.** 1977. Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts on the viral genome. Cell **12:**275–285.
6. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252:**1102–1106.
7. **Cohen, G. H.** 1972. Ribonucleotide reductase activity of synchronized KB cells infected with herpes simplex virus. J. Virol. **9:**408–418.
8. **Cohen, G. H., M. W. Facto, and M. Ponce De Leon.** 1974. Inhibition of herpes simplex virus type 2 replication by thymidine. J. Virol. **14:**20–25.

8a. **Conner, J.** Unpublished data.

9. **Conner, J., J. Macfarlane, H. Lankinen, and H. Marsden.** 1992. The unique N-terminus of the herpes simplex virus type 1 large subunit is not required for ribonucleotide reductase activity. J. Gen. Virol. **73:**103–112.
10. **Darling, A. J., E. M. Mackay, R. Ingemarson, and V. G. Preston.** 1988. Reconstitution of herpes simplex virus type 1 ribonucleotide reductase activity from the large and small subunits. Virus Genes **2:**163–176.
11. **Dutia, B. M.** 1983. Ribonucleotide reductase induced by herpes simplex virus has a virus-specified constituent. J. Gen. Virol. **64:**513–521.
12. **Dutia, B. M., M. C. Frame, J. H. Subak-Sharpe, W. N. Clark, and H. S. Marsden.** 1986. Specific inhibition of herpes simplex virus ribonucleotide reductase by synthetic peptides. Nature (London) **321:**439–441.
13. **Eriksson, S., and B.-M. Sjoberg.** 1989. Ribonucleotide reductase, p. 189–217. *In* G. Herve (ed.), Allosteric enzymes. CRC Press, Boca Raton, Fla.
14. **Frame, M. C., H. S. Marsden, and B. M. Dutia.** 1985. The ribonucleotide reductase induced by herpes simplex virus type 1 involves minimally a complex of two polypeptides (136K and 38K). J. Gen. Virol. **66:**1581–1587.

14a. **Furlong, J., et al.** Unpublished data.

15. **Furlong, J., J. Conner, J. McLauchlan, H. Lankinen, C. Galt, H. S. Marsden, and J. B. Clements.** 1991. The large subunit of herpes simplex virus type 1 ribonucleotide reductase: expression in Escherichia coli and purification. Virology **182:**846–851.
16. **Hanks, S. K., A. M. Quinn, and T. Hunter.** 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science **241:**42–51.
17. **Hattorri, M., and Y. Sakaki.** 1986. Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. **152:**232–238.
18. **Ingemarson, R., and H. Lankinen.** 1987. The herpes simplex virus type 1 ribonucleotide reductase is a tight complex of the type $\alpha_2\beta_2$ composed of 40K and 140K proteins, of which the latter shows multiple forms due to proteolysis. Virology **156:**417–422.
19. **Kamps, M. P., S. S. Taylor, and B. S. Sefton.** 1984. Direct evidence that oncogenic tyrosine kinases and cyclic AMP-dependent protein kinase have homologous ATP-binding sites. Nature (London) **310:**589–592.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **277:**680–685.
21. **Lankinen, H., E. Telford, D. MacDonald, and H. Marsden.** 1989. The unique N-terminal domain of the large subunit of herpes simplex ribonucleotide reductase is preferentially sensitive to proteolysis. J. Gen. Virol. **72:**1383–1392.
22. **Luo, J.-H., and L. Aurelian.** 1992. The transmembrane helical segment but not the invariant lysine is required for the kinase activity of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10). J. Biol. Chem. **267:**9645–9653.
23. **Luo, J.-H., C. C. Smith, M. Kulka, and L. Aurelian.** 1991. A truncated protein kinase domain of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) expressed in Escherichia coli. J. Biol. Chem. **266:**20976–20983.
24. **Maru, Y., and O. N. Witte.** 1991. The BCR gene encodes a novel serine/threonine kinase activity within a single exon. Cell **67:**459–468.
25. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
26. **Nikas, I., A. J. Darling, H. M. Lankinen, A. M. Cross, H. S. Marsden, and J. B. Clements.** 1990. A single amino acid substi-

tution in the large subunit of herpes simplex virus type 1 ribonucleotide reductase which prevents subunit interaction. J. Gen. Virol. **71**:2369–2376.
27. **Nikas, I., J. McLauchlan, A. J. Davison, W. R. Taylor, and J. B. Clements.** 1986. Structural features of ribonucleotide reductase. Protein Struct. Func. Genet. **1**:376–384.
28. **Nordlund, P., B.-M. Sjoberg, and H. Eklund.** 1990. Three-dimensional structure of the free radical protein of ribonucleotide reductase. Nature (London) **345**:593–598.
29. **Paradis, H., P. Gaudreau, B. Massie, N. Lamarche, C. Guilbault, S. Gravel, and Y. Langelier.** 1991. Affinity purification of active subunit 1 of herpes simplex virus type 1 ribonucleotide reductase exhibiting a protein kinase function. J. Biol. Chem. **266**:9647–9651.
30. **Preston, V. G., J. W. Palfreyman, and B. M. Dutia.** 1984. Identification a herpes simplex virus type 1 polypeptide which is a component of the virus-induced ribonucleotide reductase. J. Gen. Virol. **65**:1457–1466.
31. **Russo, M. W., T. J. Lukas, S. Cohen, and J. V. Stares.** 1985. Identification of residues in the nucleotide binding site of the epidermal growth factor receptor/kinase. J. Biol. Chem. **260**: 5205–5208.
32. **Seto, D.** 1990. An improved method for sequencing double stranded plasmid DNA from minipreps using DMSO and modified template preparation. Nucleic Acids Res. **18**:5905–5908.
33. **Stubbe, J.** 1989. Protein radical involvement in biological catalysis? Annu. Rev. Biochem. **58**:257–285.
34. **Stubbe, J.** 1990. Ribonucleotide reductases: amazing and confusing. J. Biol. Chem. **265**:5329–5332.
35. **Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. **185**:60–89.
36. **Swain, M. A., and D. A. Galloway.** 1986. Herpes simplex virus specifies two subunits of ribonucleotide reductase encoded by 3′-coterminal transcripts. J. Virol. **57**:802–808.
37. **Thelander, L., and P. Reichard.** 1979. Reduction of ribonucleotides. Annu. Rev. Biochem. **48**:133–158.
38. **Watson, R. J., C. M. Preston, and J. B. Clements.** 1979. Separation and characterization of herpes simplex virus type 1 immediate-early mRNAs. J. Virol. **31**:42–52.
39. **Wu, J. Y., Z.-Y. Zhou, A. Judd, C. A. Cartwright, and W. S. Robinson.** 1990. The hepatitis B virus-encoded transcriptional *trans*-activator hbx appears to be a novel protein serine/threonine kinase. Cell **63**:687–695.
40. **Wymer, J. P., T. D. Chung, Y.-N. Chang, G. S. Hayward, and L. Aurelian.** 1989. Identification of immediate-early-type *cis*-response elements in the promoter for the ribonucleotide reductase large subunit from herpes simplex virus type 2. J. Virol. **63**:2773–2784.