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Immunological Characterization of Herpes Simplex Virus Type 1 and 2 Polypeptide(s) Involved in Viral Ribonucleotide Reductase Activity

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Mammalian cells infected with herpes simplex virus (HSV) express a novel ribonucleotide reductase which is biochemically and immunologically distinct from the uninfected-cell enzyme. Using polyvalent rabbit antiserum raised against partially purified HSV type 2 reductase as well as monoclonal antibodies to HSV type 1 and HSV type 2 early antigens, we have been able to show that in both serotypes reductase activity is associated with phosphoproteins of molecular weights 144,000 and 38,000 encoded between map units 0.566 and 0.602 in the viral genomes. The major antigenic species (144,000) have been tentatively identified as HSV type 1 ICP6 and HSV type 2 ICP10.

Infection of mammalian cells with herpes simplex virus (HSV) results in the induction of high levels of ribonucleotide reductase activity (7, 10, 13). The induced enzyme is biochemically distinct from the uninfected-cell enzyme in that it is insensitive to allosteric inhibition by dTTP and dATP, does not require ATP, and responds differently to Mg^{2+}, salt, nonionic detergent, and high concentrations of dithiothreitol (9; unpublished data). A recent study reported on the partial purification of the HSV type 2 (HSV-2) reduced reductase by sedimentation in glycerol gradients and its use as an immunogen in rabbits (9). The resulting antiserum was shown to react specifically with HSV-2-infected, but not mock-infected, cells and to immunoprecipitate a major polypeptide of molecular weight 144,000 (144k) together with minor species ranging from 115k to 38k. In addition, the antiserum was capable of specifically immunoprecipitating the HSV-2 reductase (8, 9). Significant, but reduced, levels of reactivity with an HSV type 1 (HSV-1) specified antigen of 144k were also observed. Taken together, these data strongly suggested that the HSV-induced enzyme is immunologically distinct from the uninfected-cell enzyme and is very likely virally coded. Conclusive evidence in support of this hypothesis was obtained by competition experiments between rabbit serum and a monoclonal antibody specific for viral polypeptides of 144k and 38k translated in vitro from viral messages selected by DNA sequences within the *Bgl*II N fragment of the HSV-2 genome (5, 8). These experiments allowed us to map the HSV-2 reductase to a region within the HSV-2 chromosome between map units 0.566 and 0.602 (8).

In this report, we present data demonstrating that the HSV-1-induced reductase is also associated with polypeptides of 144k and 38k. Together with data of others on the location of these antigens (1, 4, 11, 12) and data on temperature-sensitive lesions affecting HSV-1 reductase (3), our results indicate that the reductase genes are colinear in HSV-1 and HSV-2. In addition, we show that in both serotypes the two polypeptides associated with reductase activity are phosphorylated. Finally, we tentatively identify one likely constituent of the viral enzyme as HSV-1 ICP6 (17) and HSV-2 ICP10 (14).

Throughout this study, BHK-21 cl.13 cells mock infected or infected with HSV-1 strain KOS or HSV-2 strain 333 (16) and harvested at 7 h postinfection were used as sources of reductase or antigens (9). The production and properties of the R1 rabbit serum specific for the HSV-2 reductase have been described previously (8, 9). Bg7 monoclonal antibody was obtained by immunizing BALB/c mice with lysates of HSV-2-infected cells prepared at 7 h postinfection (M. J. Evelegh et al., manuscript in preparation). A6 and H11 monoclonal antibodies to HSV-2 polypeptides encoded within map units 0.566 and 0.602 (5) were kindly provided by L. Goldstein; 48S and 2S monoclonal antibodies to HSV-1 ICP6 (17) were a gift from M. Zweig. Immunoprecipitation and electrophoresis of labeled polypeptides and enzymatic assays of ribonucleotide reductase were performed as described previously (8, 9).

Figure 1 illustrates the immunoprecipitation patterns obtained by reacting lysates of HSV-1 or HSV-2-infected cells with the various antibodies. All of the monoclonal antibodies were capable of immunoprecipitating a major species of 144k and a minor species of 38k. Except for monoclonal antibody 48S, which appeared to react equally well with HSV-1 and HSV-2 lysates, the other antibodies exhibited type specificity towards HSV-1 (2S) or HSV-2 (Bg7, A6 [not shown]) or had much greater affinity for HSV-2 antigens (R1, H11), in agreement with results of previous reports (5, 9, 17). No reactivity with lysates of mock-infected cells was observed with any of the antibodies (shown only for R1).

Serotype specificity was also observed in the ability of antibodies to immunoprecipitate viral reductase (Fig. 1). Antibody A6 precipitated only the HSV-2 enzyme, 2S reacted only with HSV-1, and 48S, H11, and rabbit serum precipitated both HSV-1 and HSV-2 reductase (Table 1). Of the antibodies used in the experiment depicted in Fig. 1, only Bg7 was capable of neutralizing viral reductase activity. As little as 10 μg of Bg7 immunoglobulin G (IgG) reduced HSV-2 reductase activity by ca. 60%, and nearly complete inhibition was obtained with 30 μg of IgG (Table 2). A limited amount of neutralization was also obtained with the HSV-1 enzyme, suggesting that, at least by this test, Bg7 exhibits some cross-reactivity. On the other hand, as much as 60 to 100 μg of Bg7 IgG was required to reduce the activity of the

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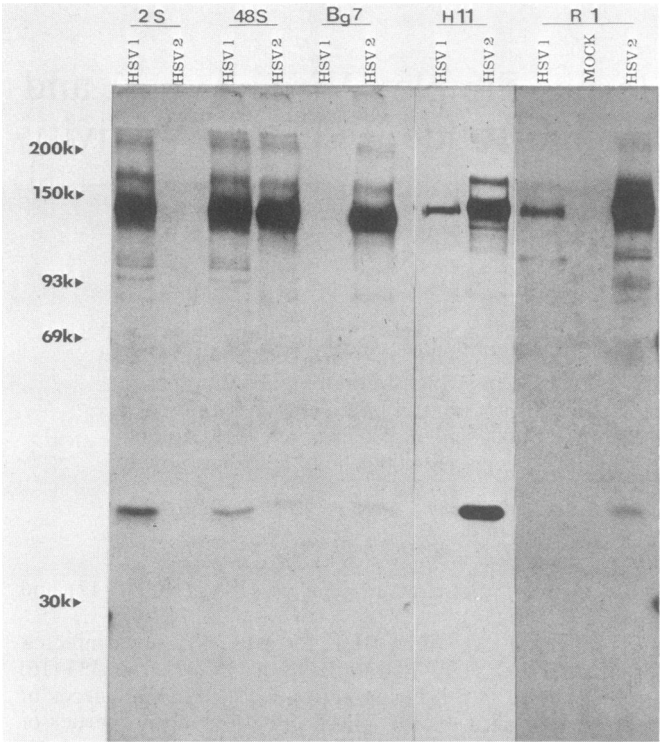

FIG. 1. Immunoprecipitation of [^{35}S]methionine-labeled antigens from HSV-infected and mock-infected cells, using monoclonal antibodies and antireductase rabbit serum. BHK-21 cl.13 cells were infected with HSV-1 or HSV-2 at a multiplicity of infection of 10 PFU per cell or mock infected, labeled with 20 μCi of [^{35}S]methionine per ml, and harvested at 7 h postinfection. Cell lysates, prepared as previously described (9), were reacted with antibodies in the presence of protein A-Sepharose, and the products of the reaction were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9).

uninfected-cell enzyme by any significant amount (25% inhibition).

Previous characterization of the 2S and 48S antibodies has established that the major species they recognize is a phosphorylated product (17). Reaction of ^{32}P-labeled HSV-2-infected cell lysates with representative antibodies indicated that the HSV-2 144k and 38k species they recognize are both

TABLE 1. Immunoprecipitation of viral ribonucleotide reductase activity by monoclonal antibodies and R1 serum

IgG[a]	Activity in immunoprecipitates of [b]:	
	HSV-1	HSV-2
2S	37.4	2.0
A6	3.1	30.2
48S	40.8	19.8
H11	57.7	52.3
R1	37.4	41.8

[a] IgG (100 μg) from monoclonal antibodies 2S, A6, 48S, and H11 and IgG (1 mg) from R1 rabbit serum were used in the reactions.

[b] Crude extracts of BHK-21 cl.13 cells, infected with HSV-1 or HSV-2 and harvested at 7 h postinfection, were reacted with IgG in the presence of protein A-Sepharose beads. After centrifugation, ribonucleotide reductase activity was measured in both supernatants and immunocomplexes as previously described (8). Enzymatic activity in the immunoprecipitates is expressed as the percentage of total activity recovered (ca. 80%).

TABLE 2. Neutralization of virus-induced reductase activity by Bg7 monoclonal antibody

Bg7 IgG (μg)	Enzyme activity (%) of [a]:		
	HSV-2	HSV-1	Mock
0	100	100	100
10	43	ND[b]	ND
30	3	62	105
60	1	ND	72
100	1	50	75

[a] Enzyme preparations from BHK-21 cl.13 cells infected with HSV-2 or HSV-1 or mock infected were incubated for 30 min at room temperature with increasing concentrations of IgG purified from Bg7 ascitic fluid. Reductase activity was then assayed and expressed as the percentage of activity measured in the reaction without IgG.

[b] ND, Not determined.

phosphorylated (Fig. 2). R1 serum also reacted with additional species, some of which were not observed in [^{35}S]methionine-labeled preparations (Fig. 1).

As already mentioned, the map location of the HSV-2 144k and 38k antigens reacting with H11, A6, Bg7, and R1 serum has been established (5, 8). It has also been shown that the two proteins are translated from two 3′ coterminal unspliced messages (12), and it is known that the corresponding region of the HSV-1 genome has a similar structure in terms of both mRNAs and polypeptides (1, 4, 11, 12). Our present data indicate that in both serotypes these polypeptides are associated with reductase activity and thus provide a further example of colinearity in the gene order of HSV-1 and HSV-2. This conclusion is in agreement with mapping data derived from studies with temperature-sensitive mutants (3).

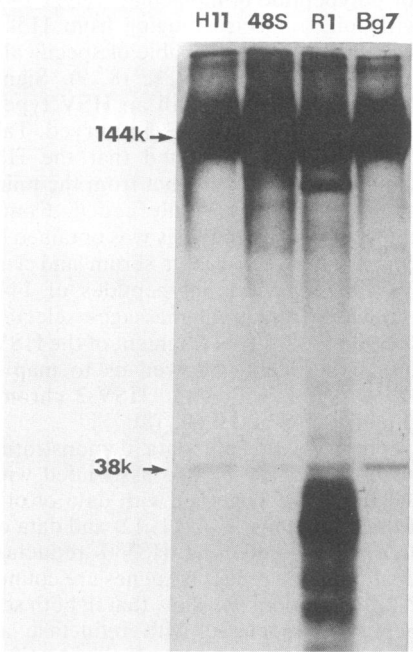

FIG. 2. Immunoprecipitation of ^{32}P-labeled antigens from HSV-2-infected cells with antibodies to viral ribonucleotide reductase. BHK-21 cl.13 cells were infected with 10 PFU of HSV-2 strain 333 per cell, labeled with 600 μCi of $^{32}PO_4$ per ml, and harvested at 7 h postinfection. Preparation of cell lysates, immunoprecipitation, and electrophoresis were done as described in the legend to Fig. 1.

Antibodies 2S and 48S have been tentatively identified as being specific for HSV-1 ICP6, based on the molecular weight and phosphorylation of the major antigen they recognize (17). Our data provide a map location and suggest a functional role for this viral gene product. The HSV-2 counterpart is likely to be ICP10, a phosphoprotein belonging to the early class of viral polypeptides (as does the reductase) and roughly mapped to the same region of the genome (5).

Questions remain as to whether the 144k and 38k polypeptides are both necessary for reductase activity and whether these polypeptides share antigenic determinants or are associated in a protein-protein complex. As described in this paper, all of the antibodies used reacted with both polypeptides, and no sera have yet been obtained which react with one but not the other polypeptide. We are thus unable at present to establish the individual role of the proteins in enzymatic activity. Equally unclear is the physical relationship between the two proteins. Sequencing of the regions encoding the 38k polypeptides in both HSV-1 and HSV-2 suggests that the two proteins are read in different frames (2; D. A. Galloway, personal communication), in agreement with unpublished data from our laboratory indicating that they have different peptide maps. This suggests that the proteins might be associated in a complex. Data on the very rapid sedimentation rate of the partially purified viral enzyme agree with this hypothesis (9); in addition, multienzyme complexes containing reductase have been identified in mammalian cells (15). Also, in nearly all procaryotic and eucaryotic systems studied, ribonucleotide reductase is comprised of two nonidentical subunits (6, 18). On the other hand, Western blot analysis of the HSV-2 144k and 38k antigens (5) indicates that they are both recognized by monoclonal antibodies after their physical separation, strongly supporting the hypothesis that they share antigenic determinants, which may be coded by a common DNA sequence present in both reading frames. We are currently attempting physical separation of the two proteins to answer both of these questions.

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