The Ribonucleotide Reductase Induced by Herpes Simplex Virus Type 1 Involves Minimally a Complex of Two Polypeptides (136K and 38K)

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SUMMARY

Herpes simplex virus type 1 (HSV-1) encodes a polypeptide of apparent mol. wt. 136 000 (Vmw136) known to be a component of the virus-specified ribonucleotide reductase. Monoclonal antibodies that precipitate this polypeptide also precipitate a polypeptide of mol. wt. 38 000 (Vmw38) from extracts of HSV-1-infected cells. The basis for this co-precipitation has been investigated using a monoclonal antibody directed against Vmw136 and an oligopeptide-induced antiserum directed against the carboxy terminus of Vmw38. We have also made use of a temperature-sensitive (ts) mutant of HSV-1 which maps within the sequences encoding Vmw136 and which induces a thermolabile ribonucleotide reductase. Our experiments show (i) Vmw136 and Vmw38 form a complex in infected cells and (ii) the mutation in the ts mutant results in the two polypeptides being unable to form the complex at the non-permissive temperature. We speculate that association of the two polypeptides is necessary for ribonucleotide reductase activity. No evidence was found for involvement of host proteins in the proposed virus-induced ribonucleotide reductase complex. The terms RR1 and RR2 are suggested for the large and small subunits of the HSV-induced enzyme.

Ribonucleotide reductase is an enzyme essential for DNA synthesis in all prokaryotic and eukaryotic cells. All ribonucleotide reductases studied so far fall into one of two classes, one having two non-identical subunits and the other only one subunit (Reichard & Ehrenberg, 1983). A number of herpesviruses induce ribonucleotide reductase activity which differs from the host cell enzyme. These include herpes simplex virus type 1 (HSV-1) (Cohen, 1972), HSV-2 (Cohen et al., 1974), pseudorabies virus (PrV) (Lankinen et al., 1982), equine herpesvirus type 1 (Cohen et al., 1977) and Epstein–Barr virus (EBV) (Henry et al., 1978). The enzymes induced by HSV-1, HSV-2 and PrV have been studied in some detail. Their susceptibility to hydroxyurea (Lankinen et al., 1982; Langelier & Buttin, 1981; Averett et al., 1983) and electron paramagnetic resonance studies on the PrV enzyme (Lankinen et al., 1982) suggest that they belong to the two-subunit class of enzyme. The HSV-1 enzyme, like all other ribonucleotide reductases, catalyses reduction of all four ribonucleotides to the corresponding deoxyribonucleotides (Averett et al., 1983; B. M. Dutia, unpublished data) and, as is known for most of the other two-subunit enzymes, utilizes nucleoside diphosphates as substrates (Averett et al., 1983).

For HSV-1 it has been shown that the enzyme is virus-coded (Dutia, 1983). The temperature-sensitive (ts) mutant of HSV-1, 17 ts VP 1207 (ts1207) has a lesion in the sequences encoding Vmw136 which results in a thermolabile ribonucleotide reductase activity and identifies Vmw136 as an essential component of the HSV-1-induced ribonucleotide reductase (Preston et al., 1984). Vmw136 has been variously designated in other publications as ICP 6 (Honess & Roizman, 1973, 1974; Showalter et al., 1981), 136K (Marsden et al., 1978), 140K (Anderson et al., 1981; Galloway et al., 1982), 144K (Huszar & Bacchetti, 1983) and 143K (Dutia et al., 1985). Monoclonal antibodies that bind to ribonucleotide reductase immunoprecipitate HSV-1 Vmw136. In addition, they precipitate HSV-1 Vmw38 (Preston et al., 1984; Bacchetti et al., 1984) and polypeptides of approximately 100 000 and 90 000 mol. wt. (Preston et al., 1984). Similar observations have been made with HSV-2-infected cells (Huszar & Bacchetti, 1983; Bacchetti et al., 1984). Furthermore, Vmw136 and Vmw38 co-purify during ribonucleotide reductase
purification from HSV-2-infected cells (Huszar et al., 1983). These data suggest that the large and small polypeptides could be associated, forming a ribonucleotide reductase complex in virus-infected cells. However, the co-precipitation data could also be explained by the two proteins possessing one or more common antigenic determinants, as has been suggested (Galloway et al., 1982). Our investigation demonstrates the existence of a complex.

Rabbits were immunized with a conjugate of bovine serum albumin (BSA) and the peptide NH₂-Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu-COOH (Cambridge Research Biochemicals, Cambridge, U.K.). This sequence represents the carboxy terminal seven amino acids of Vmw38 and of the equivalent HSV-2 polypeptide, Vmw36, as predicted from the DNA sequence (McLauchlan & Clements, 1982, 1983a). It also contains an amino terminal tyrosine to facilitate coupling of the oligopeptide to BSA (Bassiri et al., 1979). Anti-BSA antibodies were removed from the immune serum by absorption with BSA. Anti-peptide antibody was detected after 6 weeks by solid-phase radioimmunoassay and by immunoprecipitation analysis of [³⁵S]methionine-labelled extracts of BHK-21 clone 13 cells (Macpherson & Stoker, 1962) infected with HSV-1 strain 17 virus (Brown et al., 1973) or HSV-2 strain HG52 (Timbury, 1971).

Immunoprecipitations were carried out using an extraction buffer containing 0.1 M-Tris-HCl pH 8.0, 10% glycerol, 0.5% NP40, 0.5% sodium deoxycholate (Showalter et al., 1981). Under these conditions the anti-oligopeptide serum 13806 precipitated only Vmw36 from HSV-2-infected cells and Vmw38 from HSV-1-infected cells (Fig. 1, lanes 4, 16 and 24). Inclusion of 0.5% SDS in the precipitation buffer, however, resulted in the co-precipitation of Vmw138 from HSV-2-infected cells and the equivalent HSV-1 polypeptide, Vmw136, from HSV-1-infected cells (Fig. 1).
Short communication

Fig. 2. Immune precipitation of polypeptides from BHK cells infected with HSV-1 strain 17 (17+) and the mutant ts1207 by 13806 serum. Cells were infected or mock-infected at either 31 °C or 39.5 °C (permissive and non-permissive temperatures respectively of the ts mutant). Cells infected at 31 °C were labelled from 1 to 16 h post-infection with [35S]methionine using 25 μCi/ml while cells infected at 39.5 °C were labelled from 1 to 7 h post-infection using 50 μCi/ml. Extracts (EX) were made and immunoprecipitations were performed with either non-immune rabbit serum (C) or 13806 serum (Ab), with the addition of 0.5% SDS as described in the text. Polypeptides were resolved on 7.5% SDS-polyacrylamide gels. All samples were run on the same slab gel but the lanes have been realigned to place the extract immediately to the left of the lanes showing the immunoprecipitations made from it. The figure is a composite of two exposures of the same gel. The exposure for the extracts was 7 days and that for the immunoprecipitations 14 days.

1, lanes 8, 18 and 23). Higher concentrations of SDS progressively decreased the overall efficiency of the immunoprecipitation (Fig. 1, lanes 10 and 12). We suggest the explanation for these observations is that the small polypeptide was present both free and complexed with the large polypeptide in infected cells, and while the anti-oligopeptide serum can interact with free small polypeptide, the binding of the large polypeptide prevents it from doing so. Inclusion of 0.25% to 1.0% SDS partially denatures the complex without causing complete dissociation, so allowing the carboxy terminus of the Vmw38/Vmw36 polypeptide to become exposed and available to combine with specific antibody. At the higher SDS concentrations of 1% and 2% the epitope or the antibody itself may become denatured to an extent which progressively inhibits the immune interaction between them.

Strong evidence, reported below, for association of the two proteins was obtained from further studies with the HSV-1 mutant ts1207. BHK-21 cells were infected with HSV-1 parent virus or the mutant at 31 °C for 16 h and 39.5 °C for 7 h. Partially purified ribonucleotide reductase extracts were obtained by the ammonium sulphate fractionation procedure (Huszar et al., 1983) and immunoprecipitations performed as previously described (Preston et al., 1984). The results of immunoprecipitations with the anti-oligopeptide serum and a monoclonal antibody against Vmw136 (MA1100; J. W. Palfreyman, unpublished) are shown in Fig. 2 and 3 respectively. In the presence of 0-5% SDS, the anti-oligopeptide serum precipitated both Vmw38 and Vmw136 from extracts of cells infected at 31 °C with either parent virus (17+) or ts1207 mutant virus.
Fig. 3. As for Fig. 2 except that the anti-V\textsubscript{mw}136 monoclonal antibody (MA1100) was used in place of the V\textsubscript{mw}38 oligopeptide-induced antiserum. No SDS was used in the immune precipitations. Lanes 4', 5', 8' and 9' are shorter exposures of lanes 4, 5, 8 and 9 respectively. The exposures were chosen so that the density of V\textsubscript{mw}136 in lanes 3' and 9' approximated that of V\textsubscript{mw}136 in lanes 3 and 11 to show convincingly the absence of V\textsubscript{mw}38 from immune precipitations of extracts of cells infected with ts\textsubscript{1207} at 39.5 °C (lane 11) compared with cells infected at 31 °C (lanes 9 and 9') or from cells infected with the parental virus (lanes 3, 5 and 5').

They also precipitated both polypeptides from extracts of cells infected with parent virus at 39.5 °C; however, only V\textsubscript{mw}38 was precipitated from the mutant-infected cells at 39.5 °C (Fig. 2). The anti-V\textsubscript{mw}136 monoclonal antibody MA1100 also precipitated both polypeptides from extracts of cells infected with parent and mutant ts\textsubscript{1207} at 31 °C and with parent virus at 39.5 °C, but only V\textsubscript{mw}136 from extracts of cells infected with the mutant at the non-permissive temperature (Fig. 3). Thus, ts\textsubscript{1207} induced synthesis of both V\textsubscript{mw}136 and V\textsubscript{mw}38 at the non-permissive temperature but, in contrast to the results with the parental virus, the polypeptides induced at this temperature were not co-precipitated by either antibody. From these experiments we draw two conclusions. First, V\textsubscript{mw}136 and V\textsubscript{mw}38 form a complex in infected cells. Second, at the non-permissive temperature the mutation in V\textsubscript{mw}136 in ts\textsubscript{1207} results in the mutant polypeptide and V\textsubscript{mw}38 being unable to form the complex.

It is not possible to explain our observations on the basis of common antigenic determinants since the monoclonal antibody MA1100 and the anti-peptide serum cannot be directed against an epitope common to both polypeptides for the following reasons. If the co-precipitation of V\textsubscript{mw}136 and V\textsubscript{mw}38 by MA1100 was due to the monoclonal antibody being directed against a common antigenic determinant then it should precipitate V\textsubscript{mw}38 at the non-permissive temperature, since the lesion in ts\textsubscript{1207} is known to be in the sequences encoding V\textsubscript{mw}136 and not V\textsubscript{mw}38. However, it does not (Fig. 3). The only other possible explanation, that V\textsubscript{mw}38 is not synthesized in ts\textsubscript{1207}-infected cells at the non-permissive temperature or that it is in a non-precipitable form, is clearly not the case (Fig. 2). The temperature-sensitive mutation in V\textsubscript{mw}136 in ts\textsubscript{1207} therefore results in the mutant polypeptide and V\textsubscript{mw}38 being unable to form a complex at the non-permissive temperature. We do not exclude the possibility that V\textsubscript{mw}136 and V\textsubscript{mw}38
might share common epitopes but such putative common epitopes are not responsible for the observations reported here.

Also precipitated by MA1100 and to a lesser extent by the 13806 antiserum are polypeptides of mol. wt. 100000 and 90000 (Fig. 3). They were also precipitated by monoclonal antibody MA1026 directed against \( V_{mw}136 \) (Preston et al., 1984) and probably correspond to those of mol. wt. 110000 and 90000 respectively, reported to be co-precipitated by rabbit anti-ribonucleotide reductase antiserum (Huszar et al., 1983). Other experiments from our laboratory (H. S. Marsden, D. M. MacDonald & J. W. Palfreyman, unpublished) indicate that both are breakdown products of \( V_{mw}136 \). Their functional significance is not known.

To determine whether any host proteins were involved in the ribonucleotide reductase complex in HSV-infected cells, immunoprecipitations were carried out with the anti-oligopeptide 13806 serum using extracts of cells prelabelled with \([^{35}S]methionine\) from 16 h before infection up to the time of infection and also with cells labelled continuously from 16 h before infection to 16 h after infection. Fig. 4 shows that no host proteins from either cell extract were precipitated with immune sera compared with non-immune sera (compare lane 5 with lane 4, and lane 7 with lane 6). As expected, \( V_{mw}38 \) and \( V_{mw}136 \) were specifically immunoprecipitated from extracts of cells labelled continuously from 16 h before to 16 h after virus infection. We conclude that within the sensitivity of this experiment no evidence was found for any host or other virus-induced polypeptides in the complex.

Immunoprecipitations with monoclonal antibodies have previously shown that the HSV-I-encoded ribonucleotide reductase component \( V_{mw}136 \) co-precipitates with a smaller polypeptide \( V_{mw}38 \) (Preston et al., 1984; Bacchetti et al., 1984). The experiments reported here provide direct evidence that the co-precipitation is due to formation of a complex between \( V_{mw}136 \) and \( V_{mw}38 \). Furthermore, our experiments with the oligopeptide-induced antiserum 13806 identify, for the first time, \( V_{mw}38 \) as the polypeptide encoded by the mRNA which has the same 3' terminus as, but does not share coding sequences with, the mRNA encoding \( V_{mw}136 \) (McLauchlan & Clements, 1983a, b): that is, the two putative subunits of the HSV-1 ribonucleotide reductase complex are encoded by 3' co-terminal mRNAs with coding regions which do not overlap.

For HSV-2 we have no direct evidence for a complex. However, similarities between HSV-1 and HSV-2 ribonucleotide reductases detailed below strongly suggest that such a complex exists. First, monoclonal antibodies that precipitate HSV-2 ribonucleotide reductase activity precipitate \( V_{mw}138 \) and \( V_{mw}36 \) (Galloway et al., 1982; Bacchetti et al., 1984). Second, hybrid-selected translation has identified the polypeptides precipitated by these monoclonal antibodies as those encoded by the two HSV-2 3' co-terminal mRNAs equivalent to the HSV-1 locus (Anderson et al., 1981; Galloway et al., 1982). It consequently seems likely that HSV-2 has a similar ribonucleotide reductase complex to HSV-1.

Interestingly, a recent report (Gibson et al., 1984) shows significant homology between these HSV proteins and two EBV proteins encoded by tandemly arranged genes and having mol. wt. of 93000 and 34000. All the evidence thus points to a general type of herpesvirus group ribonucleotide reductase complex containing large and small subunits.

At present there is no direct evidence that \( V_{mw}38/V_{mw}36 \) is involved in ribonucleotide reductase activity. However, comparison of the amino acid sequence of HSV \( V_{mw}38 \) and \( V_{mw}36 \) with that of the \textit{Escherichia coli}-encoded ribonucleotide reductase small subunit (Carlson et al., 1984) showed a significant region of homology (J. McLauchlan & J. B. Clements, unpublished). It therefore seems likely that \( V_{mw}136/138 \) and \( V_{mw}38/36 \) have equivalent functions to the large and small bacterial subunits. Accordingly, we propose the terms RR\(_1\) and RR\(_2\) for the putative large and small subunits of herpesvirus-encoded ribonucleotide reductase subunits.

The results presented here increase the attractiveness of the herpesvirus-encoded ribonucleotide reductase as a target for antiviral chemotherapy for two reasons. First, the suggestion that there may be a general type of herpesvirus ribonucleotide reductase enzyme raises the possibility of producing an antiviral agent with activity against more than one herpesvirus type. Second, the interaction between the putative subunits may itself be a target in addition to the subunits themselves.
Fig. 4. Absence of cellular polypeptides from the HSV-coded ribonucleotide reductase complex. BHK cells were pre-labelled for 16 h before infection or pre- and post-labelled from 16 h before infection to 16 h after infection, with $^{35}$S-methionine. Cells were either mock-infected (MI, lane 1) or infected with 17syn+ (Inf, lanes 2 to 7) and extracts prepared as described (lanes 1 to 3). Extracts were precipitated with non-immune rabbit serum (C, lanes 4 and 6) or the 13806 anti-peptide antibody (Ab, lanes 5 and 7) as described in the text. Polypeptides were resolved by electrophoresis in a 5 to 12% SDS-polyacrylamide gradient gel.

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