JOURNAL OF VIROLOGY, Dec. 1984, p. 981–983
0022-538X/84/120981-03$02.00/0

Vol. 52, No. 3

Ribonucleotide Reductase of Herpes Simplex Virus Type 2 Resembles That of Herpes Simplex Virus Type 1

DEVRON R. AVERETT,* PHILLIP A. FURMAN, AND THOMAS SPECTOR
Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

Received 12 July 1984/Accepted 2 September 1984

The ribonucleotide reductase (ribonucleoside-diphosphate reductase; EC 1.17.4.1) induced by herpes simplex virus type 2 infection of serum-starved BHK-21 cells was purified to provide a preparation practically free of both eucaryotic ribonucleotide reductase and contaminating enzymes that could significantly deplete the substrates. Certain key properties of the herpes simplex virus type 2 ribonucleotide reductase were examined to define the extent to which it resembled the herpes simplex virus type 1 ribonucleotide reductase. The herpes simplex virus type 2 ribonucleotide reductase was inhibited by ATP and $MgCl_2$ but only weakly inhibited by the ATP · Mg complex. Deoxynucleoside triphosphates were at best only weak inhibitors of this enzyme. ADP was a competitive inhibitor (K'_i, 11 μM) of CDP reduction (K'_m, 0.5 μM), and CDP was a competitive inhibitor (K'_i, 0.4 μM) of ADP reduction (K'_m, 8 μM). These key properties closely resemble those observed for similarly purified herpes simplex virus type 1 ribonucleotide reductase and serve to distinguish these virally induced enzymes from other ribonucleotide reductases.

Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) induce unusual ribonucleotide reductase (ribonucleoside-diphosphate reductase; EC 1.17.4.1) activity in infected cells (3, 5, 6). This activity has been examined in relatively crude preparations of HSV-1-infected cells (5, 6) and HSV-2-infected cells (3, 6). In both cases, the kinetic properties appeared to differ from those of the cellular enzyme.

Although the reported properties of the HSV-2 ribonucleotide reductase are generally similar to those of the HSV-1 enzyme, a direct comparison of their reported kinetic properties has been hampered by the presence of significant levels of other enzymes, which rapidly degrade the substrate. A method for the efficient removal of these contaminating enzymes from ribonucleotide reductase preparations was recently reported (1, 7). When the HSV-1 ribonucleotide reductase purified by this method was characterized, the K'_m for the substrate CDP was found to be 0.65 μM for the purified enzyme, in contrast to the value of 12 μM previously reported (6). Thus, the removal of enzymes that degrade the substrate resulted in significantly different apparent kinetic properties, indicating that a direct comparison of the HSV-1 enzyme with the HSV-2 enzyme required a purified preparation.

In this paper, we report that the purification method previously used for the HSV-1 ribonucleotide reductase is equally useful for the purification of the HSV-2 ribonucleotide reductase. This HSV-2 ribonucleotide reductase preparation was studied with regard to those kinetic properties which distinguish the HSV-1 enzyme from the cellular enzyme. The purified HSV-2 enzyme was shown to have distinctive properties which are similar to those of the HSV-1 enzyme.

Unless otherwise noted, all reagents and procedures were similar to those previously reported (1). Briefly, HSV-1 ribonucleotide reductase was obtained from serum-starved BHK-21 cells after infection with HSV-2 strain 333. The cells were lysed, and the supernatant from centrifugation at $10^5 \times g$ was fractionated with 45% saturated $(NH_4)_2SO_4$. The precipitate was suspended, dialyzed, and applied to an ATP-agarose column. The eluted fractions containing ribonucleotide reductase were pooled, dialyzed against 20 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES)-sodium (pH 7.6)–2 mM dithiothreitol, and stored in 0.20-ml aliquots at −80°C. The purifed HSV-2 ribonucleotide reductase was recovered at an apparent overall yield of 110%. A final specific activity of 4.3 U mg^{-1} was obtained with 13 μM CDP as the substrate.

The removal of the interfering enzymatic activities was efficient; in the purified material the ratio of nucleoside diphosphate kinase activity to ribonucleotide reductase activity was 5, which was three orders of magnitude lower than the ratio in crude preparations. This made possible studies of the effects of nucleoside triphosphates. The ratio of CDP phosphatase activity to ribonucleotide reductase activity was 0.005. As a consequence of the removal of these interfering activities, substrate stability was greatly improved. After 10 min of incubation at 37°C, >95% of the substrate CDP remained as CDP (or dCDP) in assays containing 5 mM ATP and 6 mM $MgCl_2$, whereas >98% remained as CDP (or dCDP) in assays without ATP and $MgCl_2$.

The amount of product formed in standard assays was found to be a linear function of the enzyme concentration over a range of 0.3 to 1.8 mg/ml. Non-linearity was observed at low enzyme concentrations, as with the HSV-1-induced enzyme (1).

The amount of product formed with respect to time was not linear. The rate of decay of enzymatic activity at 37°C was similar to the rate observed for the HSV-1 ribonucleotide reductase (1). As was the case with the HSV-1 enzyme, a standard incubation time of 10 min was used to minimize the impact of this decay on assays of the HSV-2 ribonucleotide reductase.

A key characteristic of the HSV-1-induced ribonucleotide reductase is the insensitivity of the viral enzyme to inhibition by deoxynucleoside triphosphates. Therefore, the purified HSV-2 ribonucleotide reductase was examined for its sensitivity to dTTP and dATP. When a nonsaturating substrate concentration was used (2.7 μM CDP), dTTP and dATP

* Corresponding author.

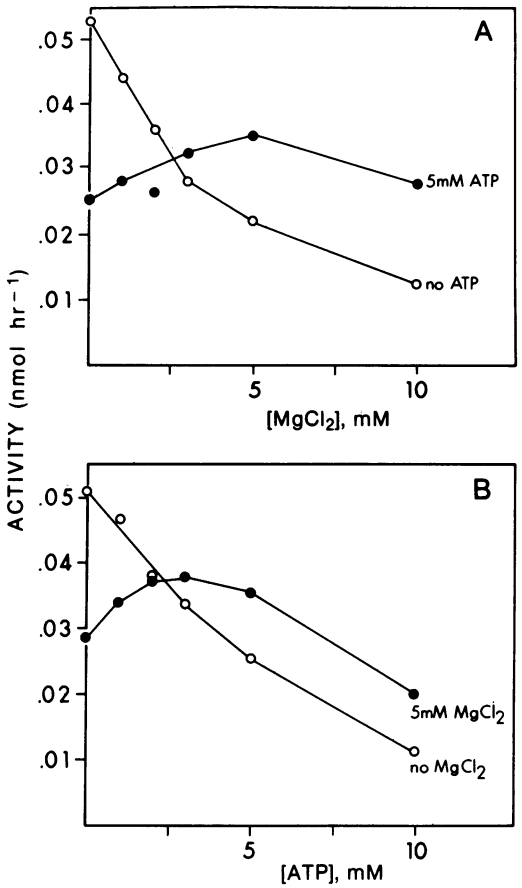

FIG. 1. Effects of ATP and $MgCl_2$ on purified HSV-2 ribonucleotide reductase. The rates of CDP reduction are plotted as a concentration of one ligand in the presence or absence of the other ligand.

inhibited the enzyme only at very high concentrations (50% inhibition concentration [I_{50}] values of 9.1 ± 2.4 mM and 5.9 ± 0.70 mM for dTTP and dATP, respectively). This resistance of the HSV-2 enzyme to inhibition by dTTP and dATP is consistent with results obtained with less-purified enzyme preparations (3, 6) and clearly differentiates the HSV-2 enzyme from the cellular ribonucleotide reductase.

Another property distinguishing the HSV-1-induced ribonucleotide reductase is its inhibition by $MgCl_2$ and ATP. The purified HSV-2 ribonucleotide reductase was tested for its response to ATP and $MgCl_2$, both alone and in combination. The HSV-2 ribonucleotide reductase was inhibited by ATP alone (I_{50}, 5.7 ± 0.32 mM) and by $MgCl_2$ alone (I_{50}, 3.7 ± 0.11 mM). However, in each case the inhibition could be partially reversed, either by the addition of $MgCl_2$ to reactions containing ATP or by the addition of ATP to reactions containing $MgCl_2$ (Fig. 1). This effect has been observed with the HSV-1 ribonucleotide reductase and is attributed to the formation of a less inhibitory ATP · Mg complex (1). The HSV-2 enzyme is thus qualitatively similar to the HSV-1 enzyme, although the two enzymes exhibit slightly different sensitivities to $MgCl_2$ and ATP. The purified HSV-2 enzyme is clearly distinguishable from the cellular enzyme, which requires ATP as an allosteric activator (2).

The kinetic relationships between representative purine and pyrimidine substrates were studied. Competitive inhibition of CDP reduction by ADP was observed (Fig. 2). The apparent K_i [K_i'] was 11 ± 0.97 μM for ADP, and the apparent K_m [K_m'] for CDP was 0.49 ± 0.044 μM. Conversely, CDP was found to be a competitive inhibitor of ADP reduction. The K_m' for ADP was 7.8 ± 1.6 μM, with an apparent V_{max} [V_{max}'] similar to the V_{max}' observed for CDP; the K_i' for CDP was 0.42 ± 0.078 μM. The good agreement of the CDP K_m' with the CDP K_i' and of the ADP K_m' with the ADP K_i' resembles the pattern observed with the HSV-1-induced enzyme and suggests the reduction of both ADP and CDP at a common site. Also, the kinetic constants for ADP and CDP with the HSV-2 enzyme were quantitatively similar to those found with the HSV-1 enzyme (1). These kinetic constants and patterns of substrate interactions are clearly different from those reported for the eucaryotic enzyme from a variety of sources (2).

The removal of interfering enzymatic activities allowed the unequivocal examination of a number of key kinetic properties which can distinguish the HSV-1 and HSV-2 ribonucleotide reductases from the ribonucleotide reductase found in uninfected cells. Like the HSV-1 enzyme, the ribonucleotide reductase from HSV-2-infected cells was only weakly inhibited by deoxynucleoside triphosphates. Also, the HSV-2 ribonucleotide reductase was inhibited by ATP alone or $MgCl_2$ alone and only weakly inhibited by the ATP · Mg complex. The HSV-1 enzyme was previously shown to be competitively inhibited by alternative substrates and had very low K_m' values for GDP, ADP, and CDP (1). The present work shows that the HSV-2 ribonucleotide reductase has similar kinetic properties. The quantitative agreement of the kinetic constants reported here with those reported previously (1) serves as a sensitive indicator of the functional similarity between the ribonucleotide reductases induced upon infection by HSV-1 and HSV-2. Thus, although small differences may exist, the HSV-2 ribonucleotide reductase closely resembles the HSV-1 ribonucleotide reductase. Furthermore, the key features examined here place both these viral enzymes in a category which is clearly distinct from other ribonucleotide reductases.

We thank Carol Lubbers for providing HSV-2-infected BHK cells.

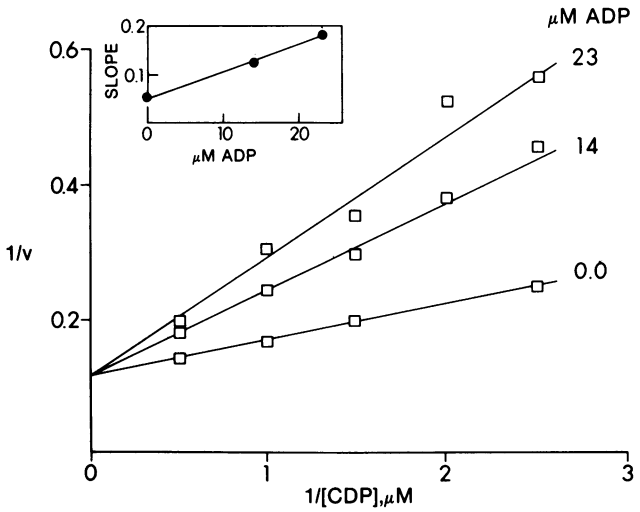

FIG. 2. Competitive inhibition of CDP reduction by ADP. Reaction mixtures contained 200 mM HEPES (pH 8.1), 10 mM dithiothreitol, and the indicated concentrations of [^{14}C]CDP and ATP. The kinetic constants are given in the text.

LITERATURE CITED

1. **Averett, D. R., C. Lubbers, G. B. Elion, and T. Spector.** 1983. Ribonucleotide reductase induced by herpes simplex type 1 virus: characterization of a distinct enzyme. J. Biol. Chem. **258:**9831–9838.
2. **Hunting, D., and J. F. Henderson.** 1982. Models of the regulation of ribonucleotide reductase and their evaluation in intact mammalian cells. Crit. Rev. Biochem. **13:**325–348.
3. **Huszar, D., and S. Bacchetti.** 1981. Partial purification and characterization of the ribonucleotide reductase induced by herpes simplex virus infection of mammalian cells. J. Virol. **37:**580–588.
4. **Huszar, D., B. Beharry, and S. Bacchetti.** 1983. Herpes simplex virus-induced ribonucleotide reductase: development of antibodies specific for the enzyme. J. Gen. Virol. **64:**1327–1335.
5. **Langelier, Y., and G. Buttin.** 1981. Characterization of ribonucleotide reductase induction in BHK-21/C13 syrian hamster cell line upon infection by herpes simplex virus (HSV). J. Gen. Virol. **57:**21–31.
6. **Ponce de Leon, M., R. J. Eisenberg, and G. H. Cohen.** 1977. Ribonucleotide reductase from herpes simplex virus (type 1 and 2) Infected and uninfected KB cells: properties of the partially purified enzymes. J. Gen. Virol. **36:**163–173.
7. **Spector, T., and D. R. Averett.** 1983. A simple method to purify ribonucleotide reductase. Anal. Biochem. **135:**469–470.