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Induction of a New Ribonucleotide Reductase After Infection of Mouse L Cells with Pseudorabies Virus

HILKKA LANKINEN,[1] ASTRID GRÄSLUND,[2] AND LARS THELANDER[1]*

Medical Nobel Institute, Department of Biochemistry, Karolinska Institute, S-104 01 Stockholm,[1] and Department of Biophysics, University of Stockholm, Arrhenius Laboratory, S-106 91 Stockholm,[2] Sweden

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The mammalian ribonucleotide reductase consists of two nonidentical subunits, proteins M1 and M2. M1 binds nucleoside triphosphate allosteric effectors, whereas M2 contains a tyrosine free radical essential for activity. The activity of ribonucleotide reductase increased 10-fold in extracts of mouse L cells 6 h after infection with pseudorabies virus. The new activity was not influenced by antibodies against subunit M1 of calf thymus ribonucleotide reductase, whereas the reductase activity in uninfected cells was completely neutralized. Furthermore, packed infected cells (but not mock-infected cells) showed an electron paramagnetic resonance spectrum resembling, but clearly different from, the electron paramagnetic resonance spectrum of the tyrosine free radical of subunit M2 of the cellular ribonucleotide reductase. These data give conclusive evidence that on infection, herpesvirus induces a new or modified ribonucleotide reductase. The virus-induced enzyme showed the same sensitivity to inhibition by hydroxyurea as the cellular reductase. The allosteric regulation of the virus enzyme was completely different from the regulation of the cellular reductase. Thus, CDP reduction catalyzed by the virus enzyme showed no requirement for ATP as a positive effector, and no feedback inhibition was observed by dTTP or dATP. The virus reductase did not even bind to a dATP-Sepharose column which bound the cellular enzyme with high affinity.

To make DNA, cells need a balanced supply of the four deoxyribonucleoside triphosphates. These are synthesized by a direct reduction of the corresponding ribonucleotides in a reaction catalyzed by ribonucleotide reductase. This enzyme therefore occupies a key position in the sequence of reactions leading to DNA replication and cell division (for a review, see reference 17). In mammalian cells ribonucleotide reductase consists of two nonidentical subunits called proteins M1 and M2 with molecular weights of 168,000 and 110,000, respectively. M1 was purified to homogeneity from calf thymus and shown to bind nucleoside triphosphate effectors (16). The detailed allosteric regulation of the calf thymus enzyme has been worked out (7). M2, which still is far from pure, is similar to the corresponding subunit in the ribonucleotide reductase from *Escherichia coli* in that it contains a tyrosine free radical essential for activity (1; A. Gräslund, A. Ehrenberg, and L. Thelander, submitted for publication).

Infection of *E. coli* by the bacteriophage T4 induces a virus-specific ribonucleotide reductase with allosteric properties altered to fit the specific requirements of viral DNA synthesis (17). A similar induction of a new or modified ribonucleotide reductase has been suggested for herpesvirus-infected cells. This is based on the observation that DNA synthesis in cells infected by herpes simplex virus is not influenced by levels of thymidine in the medium completely blocking DNA synthesis in the uninfected cell (5). It is known that the addition of thymidine to cells blocks DNA synthesis by starving the cells for dCTP since high dTTP pools shut off CDP reduction by allosteric inhibition of the cellular ribonucleotide reductase (2). After herpesvirus infection, cells have very high pools of dTTP and still make DNA, indicating a modified or new ribonucleotide reductase (10). This is further supported by the recent observations that after herpesvirus infection, the activity of ribonucleotide reductase in a cell extract is resistant to inhibition by dATP, which is a very potent inhibitor of the reductase in uninfected cells (9, 12).

Another evidence for a herpesvirus-specific ribonucleotide reductase is that DNA synthesis in cells infected by equine herpes type I or Epstein-Barr virus is reported to be resistant to hydroxyurea (8), a drug known to interact specifically with the free radical of M2, thereby inhibiting the cellular reductase (1). However, DNA synthesis in herpes simplex virus-infected cells is not resistant to hydroxyurea (12, 13, 18).

We have measured the activity of ribonucleotide reductase in extracts from mouse L cells in the stationary phase of growth and found a 10-fold increase in activity 6 h after infection. Most of this activity was due to a new, virus-induced ribonucleotide reductase as shown by immunochemical technique and electron paramagnetic resonance (EPR) spectroscopy. The virus-induced reductase showed the same sensitivity to inhibition by hydroxyurea as the cellular enzyme. In contrast, the allosteric regulation of the virus-induced reductase seemed to be quite different from the regulation of the cellular enzyme.

MATERIALS AND METHODS

Cells and virus. Mouse fibroblast L cells (NCTC clone 929), rabbit kidney (RK) cells (LLC-RK_1) and pseudorabies (PR) virus (VR-135) were all obtained from the American Type Culture Collection. Monolayer cell cultures were grown at 37°C in Dulbecco modified Eagle medium, and suspension cultures were grown in spinner bottles (Bellco Glass, Inc.) in Dulbecco suspension medium fortified with nonessential amino acids (Flow Laboratories, Inc.). Both media contained 10% heat-inactivated horse serum (30 min at 56°C) and antibiotics. Virus titers were determined by plaque assays on RK or L cells. Routinely, RK cells were used since they gave larger plaques and also the titers were 20 times higher than on L cells. However, all titers given in this paper were recalculated to the L cell values, that is, the RK cell value divided by 20, unless stated otherwise.

Virus preparation. Virus from one plaque was used to infect one 150-mm dish containing a confluent monolayer of RK cells. After incubation in medium containing 2% serum at 37°C for 72 h, the cells were scraped from the dish into the medium, and virus was released by sonication. This lysate, having a titer of 5×10^8 PFU/ml on RK cells, was used as stock for virus preparations.

To prepare virus, RK cell monolayers were infected at a multiplicity of 0.1 PFU/cell. The cells were incubated at 37°C for 48 h, scraped from the dishes, collected by centrifugation (5 min at $1,200 \times g$), and finally resuspended in a small volume of supernatant medium. Virus was released by sonication. The rest of the supernatant medium was centrifuged for 90 min at $20,000 \times g$ to collect virus released into the medium during the incubation (about 50%). The pellet was resuspended in a small volume of medium and sonicated. Titers of 2.5×10^9 PFU/ml were obtained for the combined sonicates. The virus was kept at −70°C.

Preparation of extracts from virus-infected cells. (i) Monolayer cultures. Confluent monolayers of mouse L cells (2.5×10^7 cells per 150-mm dish) were infected at a multiplicity of infection of 5 PFU/cell. Virus was absorbed for 90 min at 37°C in 1 ml of isotonic Tris-saline containing 0.1% serum; then 20ml of medium containing 2% serum was added, and incubation was continued for 4.5 h. The medium was removed, and each dish was washed twice with 20 ml of ice-cold Tris-saline and once with 10 ml of ice-cold 20 mM 4-(2-hydroxyethyl)-1-piperazinesulfonic acid buffer (pH 7.5)–10 mM $MgCl_2$–2 mM dithiothreitol. The dishes were left in a vertical position for 10 min, residual buffer was removed, and then the cells were scraped from the dishes and transferred to a tight pestle glass homogenizer. The suspension was rapidly frozen in liquid nitrogen, thawed, and homogenized. After centrifugation in a Sorvall SS-34 rotor at 15,000 rpm for 30 min at 0°C, the supernatant solution was rapidly frozen and kept at −70°C. The protein concentration in the extracts varied from 2 to 4 mg/ml.

Mock infection was also made with cells in the stationary phase of growth, and extracts were prepared as described above.

(ii) Suspension cultures. Suspension cultures of L cells in the stationary phase of growth were collected by centrifugation, resuspended in medium without serum at a concentration of 10^7 cells per ml, and infected at a multiplicity of 5 PFU/cell. After 90 min of adsorption at 37°C, medium containing 2% serum was added to give a final cell concentration of 10^6 cells per ml. After 2.5 h of incubation the cells were collected by centrifugation, washed once with ice-cold Tris-saline, transferred to a glass homogenizer, and extracted as described above.

Preparation of extracts from uninfected cells. Monolayers of L cells in the mid-log phase of growth were harvested and extracted as described above.

Partial purification of ribonucleotide reductase. Solid ammonium sulfate (277 mg/ml) was added under stirring at 0°C to crude extracts from infected or uninfected cells (5 to 20 mg of protein). After 30 min of incubation the precipitate was collected by centrifugation and redissolved in a small volume of 50 mM Tris-Cl buffer (pH 7.6). The protein solution was then passed through a Sephadex G-50 column (sample/column volume ratio, 1:10) equilibrated with the same buffer. Routinely this gave a doubling of the specific activity of ribonucleotide reductase with a yield of about 50%. Also, the solution was concentrated with respect to protein and freed of nucleotides. These extracts were used in the nucleotide effector experiments.

Protein determination. The protein concentration of cell extracts was determined by the Coomassie brilliant blue G-250 method calibrated by the Lowry method as modified by Jovin, with bovine serum albumin as a standard as described previously (6).

Enzyme assay. Ribonucleotide reductase activity was measured by determining the reduction of [^{3}H]CDP as described previously (6). The following reagents were incubated at 37°C for 30 min in a final volume of 150 μl: 75 nmol of [^{3}H]CDP (specific activity, 97,000 cpm/nmol), 6 μmol of 4-(2-hydroxyethyl)-1-piperazinesulfonic acid buffer (pH 7.6), 0.6 μmol of ATP, 1.5 μmol of $MgCl_2$, 1.5 μmol of dithiothreitol, 15 μmol of KCl, 3 nmol of $FeCl_3$, and 50 to 100 μl of cell extract. One unit of ribonucleotide reductase activity is defined as the amount of enzyme which catalyzes the formation of 1 nmol of dCDP per min at 37°C.

Binding of ribonucleotide reductase activity to dATP-Sepharose. Partially purified extracts from infected and uninfected cells (0.5 and 1.3 mg of protein, respectively, with specific activities of 42 and 37 mU/mg in 500 μl of 50 mM Tris-Cl buffer, pH 7.6) were loaded on two 0.5- by 1.3-cm columns of dATP-Sepharose (6) equilibrated with the same buffer. Each extract was passed through the column twice at 4°C, and then the

eluates and starting materials were assayed for protein and enzyme activity.

EPR measurements. EPR is a spectroscopic technique uniquely applied to study atomic or molecular systems containing unpaired electrons, such as free radicals. The EPR spectrum describes the absorption of energy in the sample as a function of an external magnetic field. For technical reasons it is generally recorded as the first derivative of the absorption. The intensity of the EPR absorption (calculated as a double integral of the first-derivative spectrum) is proportional to the number of unpaired electrons in the sample. The pattern of spectral lines from a free radical (hyperfine pattern) reflects the arrangement of magnetic nuclei surrounding the electron, since these neighboring magnetic nuclei give rise to internal magnetic fields which add to or subtract from the externally applied field. The preparation of cells for EPR measurements was described earlier (1). The EPR experiments were performed on a Varian V-4502 spectrometer, equipped with 100-kHz field modulation, a 4-in. (ca. 10-cm) magnet and a V-4531 multipurpose cavity. For temperature control an Oxford Instrument helium flow cryostat (ESR-9) was used. A Varian 620-i computer was attached on line to the spectometer for signal averaging to improve the signal/noise ratio and for the subtraction of spectra.

RESULTS

Activity of ribonucleotide reductase in cells after infection with pseudorabies virus. Monolayers of mouse L cells in the stationary phase of growth were infected with PR virus, and the activity of ribonucleotide reductase was measured in cell extracts from 0 up to 8 h postinfection (Fig. 1). There was an increase in activity already 2 h postinfection as compared with mock-infected cells, and the activity reached a maximum after 6 h and then started to decrease again. The activity of uninfected cells remained very low during the whole experiment. After 6 h the activity in infected cells was 10-fold higher than that in uninfected cells. Ribonucleotide reductase activity 6 h postinfection increased in cell extracts with increasing multiplicities of infection, reaching a maximal value at 5 PFU/cell; therefore, this value was used throughout this work.

The same increase and variation of ribonucleotide reductase activity with time after infection was observed in suspension cultures, but activity reached a maximum already 4 h postinfection (data not shown).

Ribonucleotide reductase activity in extracts from infected cells is not inhibited by antibodies against subunit M1 of calf thymus ribonucleotide reductase. Ribonucleotide reductase activity in extracts from infected and uninfected cells was tested for cross-reactivity to mouse monoclonal antibodies against the M1 subunit of calf thymus ribonucleotide reductase (Y. Engström and L. Thelander, unpublished results). The activity from infected cells was not at all influenced by

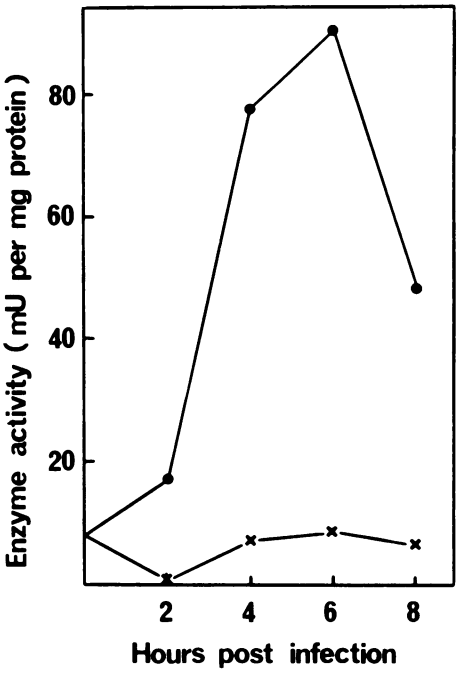

FIG. 1. Ribonucleotide reductase activity in extracts from PR virus-infected (●) and mock-infected (×) mouse L-cell monolayers. Each point gives the mean value of two activity measurements, using 50 and 100 μl of extract containing 2 to 3 mg of protein per ml. The multiplicity of infection was 5 PFU/cell.

an amount of antibody which completely neutralized the activity in an extract from uninfected cells (Fig. 2). The same amounts of antibody from nonimmunized mice had no inhibitory effect on either extract. Similar results were obtained by using rabbit antibodies against the calf thymus protein M1. The data clearly show that a new or modified M1 subunit of ribonucleotide reductase is induced in cells on infection by PR virus.

PR virus-infected cells contain increased levels of a specific tyrosine free radical EPR signal different from but resembling the EPR signal observed in mouse cells overproducing subunit M2. EPR spectroscopy at low temperature (77 K) has shown that the B2 subunit of ribonucleotide reductase from *E. coli* contains a stable free radical essential for enzyme activity. To investigate the origin of this radical, isotopic substitution experiments were performed, whereby the magnetic properties of certain nuclei possibly in the neighborhood of the unpaired electron were changed. By analysis of the EPR spectra of ribonucleotide reductase-overproducing bacteria grown in media containing different labeled compounds, the radical was shown to be localized to a tyrosine residue, probably formed by

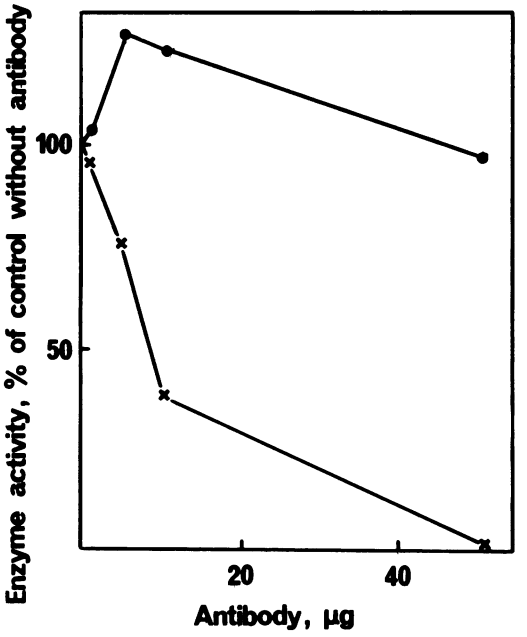

FIG. 2. Inhibition of ribonucleotide reductase in extracts from PR virus-infected and uninfected cells by mouse monoclonal antibodies directed against the M1 subunit of calf thymus ribonucleotide reductase. A constant amount of extract from infected cells (300 μg of protein; ●) and from uninfected exponentially growing cells (400 μg of protein; ×) was incubated in two series of mixtures for 30 min at 0°C with the indicated amounts of antibody (clone AC1) and then assayed for ribonucleotide reductase activity. The specific activities were 19 and 8 mU/mg for infected and uninfected cell extracts, respectively.

the loss of an electron from the aromatic ring system (15).

Recently we found that packed hydroxyurea-resistant mouse fibroblast 3T6 cells overproducing the M2 subunit give rise to a characteristic EPR spectrum very similar to that of the B2 subunit of *E. coli* (1). This EPR signal is not detected in normal hydroxyurea-sensitive parent mouse fibroblasts. Again, isotopic substitution experiments and EPR spectral analysis have shown the radical to be localized to a tyrosine residue also in the M2 subunit (Gräslund et al., submitted for publication). The EPR spectra of the bacterial and mammalian reductases at 77 K have the same basic hyperfine patterns and are similar, but not identical. The differences were interpreted as being due to slightly different sterical conformations of the tyrosine residues at the active site in the two systems. We centrifuged PR virus-infected L cells as well as mock-infected cells in two EPR tubes and recorded their EPR spectra at 32 K (Fig. 3a and b, respectively). Fig. 3c shows a background signal, mainly arising from the quartz of the sample tube, which interferes at the center of the signals at these conditions of high sensitivity of the spectrometer.

The mock-infected cells (Fig. 3b) gave a broad structureless EPR signal, whereas the PR virus-infected cells (Fig. 3a) showed a signal very similar to the ones of the M2 and B2 specific tyrosine radicals. The excess of this radical is at least threefold in the PR virus-infected cells as compared with the mock-infected ones (judged from the EPR signal amplitudes). EPR recordings at 77 K gave spectra with less hyperfine structure resolution. Hydroxyurea destroys the EPR signal of the PR-infected cells as well as the signal of the overproducing 3T6 cells (1).

Isotope substitution experiments in which the cells were grown in a medium containing specifically deuterium-labeled tyrosine instead of normal tyrosine showed that also the virus-induced free radical was localized to a tyrosine residue (data not shown).

Figure 4a shows the EPR spectrum of the PR virus-induced radical signal, where the appropriate background signal of the mock-infected cells has been subtracted. Figure 4b shows the corresponding M2 signal from overproducing cells, from which the wild-type cell background has been subtracted (1). The two spectra have the same basic hyperfine pattern, but show significant differences. These are probably due to different sterical conformations of the tyrosine residues like previously observed differences between the tyrosine radicals of ribonucleotide reductases from different species (Gräslund et al., submitted for publication). These data show conclusively that a new or modified M2 subunit is induced in cells after infection with PR virus.

Properties of the PR virus-induced ribonucleotide reductase. (i) Inhibition by hydroxyurea. In Fig. 5 the effects of hydroxyurea on ribonucleotide reductase activity in extracts from infected cells are compared with the effects on an extract from uninfected cells. The activity in both extracts showed a similar sensitivity to inhibition by hydroxyurea with a 50% inhibition at about 0.5 mM concentration of the drug.

(ii) Resistance to inhibition by dTTP. Ribonucleotide reductase activity (CDP reduction) was measured in partially purified extracts (see above) from infected and uninfected cells in the presence of both ATP and dTTP as effectors (Fig. 6a). As expected, the activity in extracts from uninfected cells was almost completely inhibited by 1 mM dTTP in the presence of 3 mM ATP. In contrast, the activity in extracts from infected cells was not influenced by this combination of effectors. This was not due to breakdown of the dTTP during the incubation, since chromatography on polyethyleneimine plates of

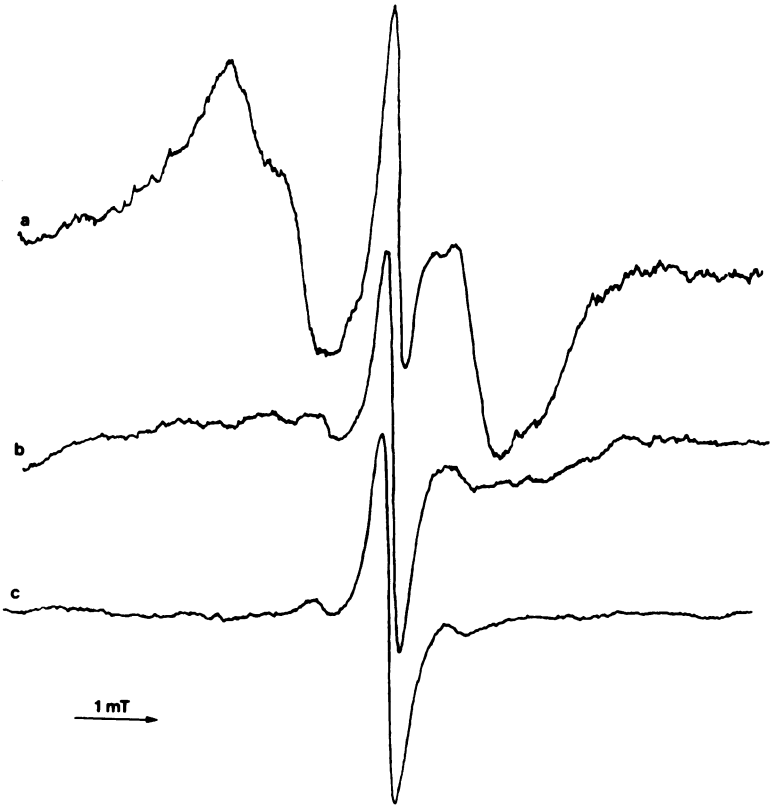

FIG. 3. EPR spectra at 32 K of packed samples of (a) PR virus-infected mouse L cells, (b) mock-infected mouse L cells, and (c) distilled water to give a background signal. In a and b 140 μl of a cell suspension containing 5×10^7 cells was used. The microwave power was 4.6 mW, and the modulation amplitude was 0.25 mT. For b and c the spectrometer amplification was ½ and ⅓, respectively, of that of a.

deproteinized samples of an incubation mixture containing [^{3}H]dTTP showed that more than 90% of the added dTTP was still intact after 30 min of incubation at 37°C.

(iii) Resistance to inhibition by dATP. The influence of dATP on CDP reduction catalyzed by a partially purified ribonucleotide reductase from infected and uninfected cells is shown in Fig. 6b. The assays were made in the presence of 3 mM ATP and increasing amounts of dATP. At 0.1 mM dATP the ribonucleotide reductase activity of uninfected cells showed a 60% inhibition as compared with a control without dATP, whereas the activity of infected cells was not affected by this concentration of deoxyribonucleotide.

The resistance of the virus-induced ribonucleotide reductase to dATP inhibition was further investigated by measuring the binding of the enzyme to an affinity chromatography column of dATP-Sepharose (1). Partially purified extracts from infected and uninfected L cells were passed through columns of dATP-Sepharose, and enzyme activity was assayed before and after passage of the columns (see above). More than 90% of the ribonucleotide reductase activity from uninfected cells bound to the affinity column under these conditions, whereas around 70% of the activity from infected cells were recovered in the eluate. In repeated attempts varying the experimental conditions we have never been able to bind the PR virus-induced ribonucleotide reductase to dATP-Sepharose, indicating a lowered or totally lost affinity to dATP.

(iv) Effects of ATP in the enzyme assay. Calf thymus ribonucleotide reductase shows an absolute requirement for ATP as the positive effector for the CDP reduction (7). In Fig. 7 the effects of ATP on the reduction of CDP catalyzed by partially purified extracts from infected and uninfected cells are shown. As expected the cellular ribonucleotide reductase showed an absolute requirement for ATP, with maximal activity around 5 mM ATP. In contrast, ATP in concentrations from 0 to 10 mM neither stimulated nor

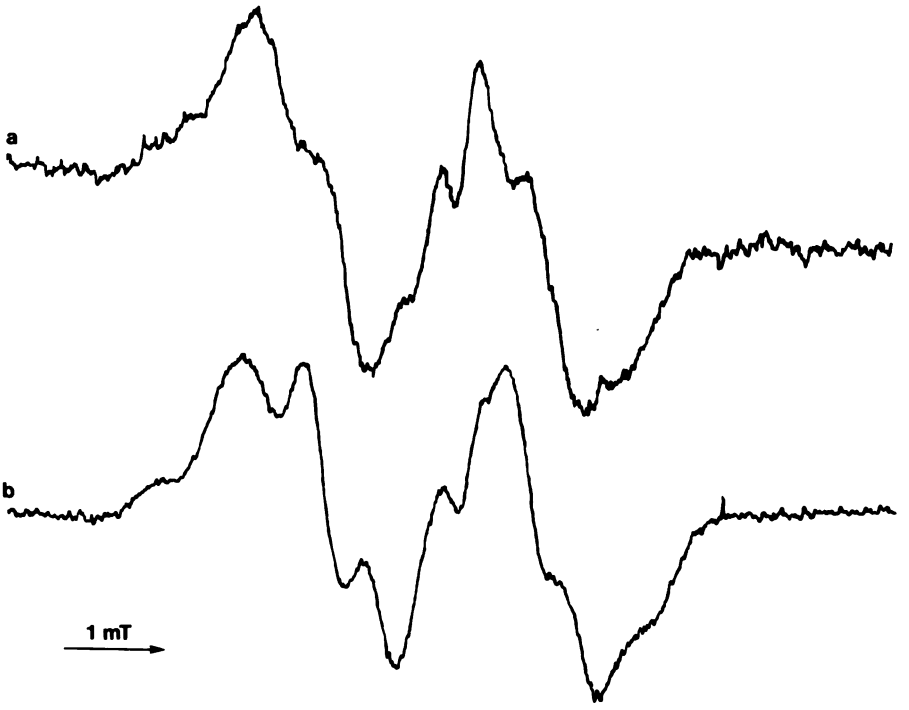

FIG. 4. EPR spectra at 32 K of packed samples of (a) PR virus-infected mouse L cells and (b) hydroxyurea-resistant, M2-overproducing mouse fibroblast 3T6 cells. Appropriate background spectra of mock-infected mouse L cells and wild-type mouse fibroblast cells, respectively, were subtracted. The microwave power was 4.6 mW in a and 1.2 mW in b. The modulation amplitude was 0.25 mT for both spectra.

inhibited the reductase activity of infected cells. In all of these experiments the concentration of magnesium ions was always kept at least twice as high as the concentration of nucleoside triphosphate.

DISCUSSION

It was reported by Kaplan already in 1964 that DNA synthesis in cells infected by PR virus is unaffected by concentrations of thymidine in the medium inhibiting DNA synthesis in uninfected cells (11). This observation was extended to herpes simplex virus by Cohen, who also suggested that the resistance to thymidine is due to a virus-induced ribonucleotide reductase being refractory to feedback inhibition by dTTP (5). Ribonucleotide reductase would then add to several other herpesvirus-induced enzymes related to DNA synthesis, such as DNA polymerase (19) and deoxypyrimidine kinase (4). Several attempts to purify a herpesvirus-induced ribonucleotide reductase have been reported, but with limited success (9, 14). One problem has been the lack of knowledge of the host cell ribonucleotide reductase due to difficulties in the purification of this enzyme from mammalian cells. We have now succeeded in purifying and characterizing the ribonucleotide reductase from calf thymus (6, 7, 16), and with this background information we decided to make a close study of the reductase activity in herpesvirus-infected cells.

The antibody inhibition data together with the results of the EPR studies presented in this paper show for the first time in a conclusive way that a new, virus-specific ribonucleotide reductase is induced in cells after herpesvirus infection. By choosing cells in the stationary phase of growth, the activity of the cellular reductase was very low, and most of the activity after infection was virus specific (Fig. 2). We cannot be certain that the enzyme is virus coded, since virus infection could also induce a modification of the cellular M1 and M2 subunits. However, this was considered less likely since rabbit antibodies directed against a variety of antigenic determinants of the cellular protein M1 failed to inhibit the reductase activity after virus infection.

The presence of a new type of EPR signal in PR virus-infected cells resembles the situation in bacteriophage T4 infected *E. coli*. T4 induces a new ribonucleotide reductase different from the host cell enzyme with respect to both subunits, and the tyrosine free radical of the T4 reductase gives an EPR signal resembling, but different

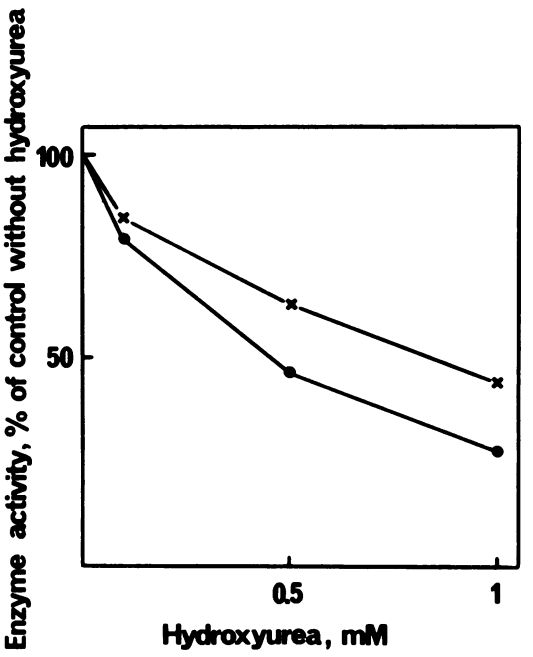

FIG. 5. Inhibition by hydroxyurea of ribonucleotide reductase activity in extracts from PR virus-infected (●) and uninfected (×) mouse L cells. The extract from infected cells had a specific activity of 40 mU/mg, and the extract from uninfected cells was prepared from cells in the mid-log phase and had a specific activity of 33 mU/mg; 100% corresponded to 0.14 and 0.10 nmol of dCDP per 30 min for infected and uninfected cell extracts, respectively.

from, the one of the *E. coli* enzyme (M. Sahlin, A. Gräslund, A. Ehrenberg, and B.-M. Sjöberg, J. Biol. Chem., in press). In T4-induced reductase the difference in the EPR signal compared with that of the host cell can be explained by a small variation in the angle of the aromatic ring in relation to the β-methylene group of the active tyrosine residue, since the hyperfine structure of the EPR spectrum is critically dependent on this angle. The variation in angle in turn probably reflects differences in the conformation of the proteins. A similar explanation for the differences observed between the EPR spectra of the PR virus induced and the host cell reductase is most likely.

Hydroxyurea has been shown to inactivate ribonucleotide reductase by scavenging the tyrosine free radical (1, 17). The similar sensitivity to inhibition by hydroxyurea of the virus induced and cellular reductases therefore indicated similar free radical structures in the active sites, and this was indeed confirmed by the EPR studies.

Our demonstration of the lack of feedback inhibition of the virus reductase by dTTP or dATP confirms data from other laboratories (5, 9, 12, 14) and suggests that herpesvirus might be similar to bacteriophage T4 in lacking negative allosteric effectors of ribonucleotide reductase (17). In contrast to Huszar and Bacchetti (9) we found no inhibition of the virus reductase-catalyzed CDP reduction by ATP. In parallel to observations with the *E. coli* ribonucleotide reductase (3), we think that the inhibition ob-

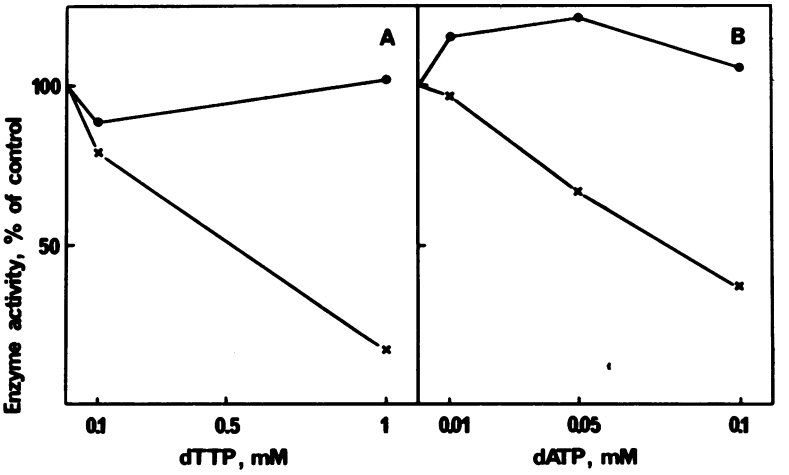

FIG. 6. Activity of ribonucleotide reductase in partially purified extracts from infected (●) and exponentially growing uninfected (×) cells assayed in the presence of (A) dTTP and (B) dATP. All incubation mixtures contained 3 mM ATP, 10 mM $MgCl_2$, and the indicated concentrations of deoxyribonucleotide. The control was incubated in the absence of deoxyribonucleotide. The specific activity of the extract from infected cells was 37 mU/mg; 100% corresponded to 0.27 nmol of dCDP per 30 min. The corresponding figures for uninfected cells were 21 mU/mg and 0.12 nmol of dCDP per 30 min.

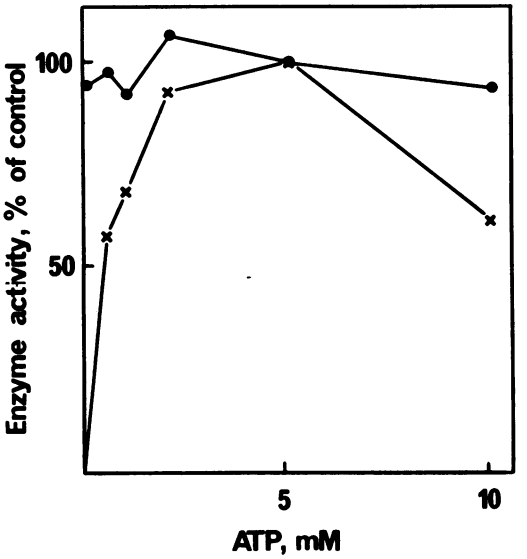

FIG. 7. Activity of ribonucleotide reductase in partially purified extracts from infected (●) and exponentially growing uninfected (×) cells in the presence of ATP. The reduction of CDP was assayed with increasing amounts of ATP; 100% is the activity obtained in the presence of 5 mM ATP. $MgCl_2$ was always added to give a concentration twice the concentration of ATP. The specific activity of the extract from infected cells was 74 mU/mg and 100% corresponded to 0.41 nmol of dCDP per 30 min. The corresponding figures for uninfected cells were 29 mU/mg and 0.11 nmol of dCDP per 30 min.

served in their studies might be due to lack of magnesium ions at high ATP concentrations since in our experiments we always added ATP as the magnesium salt.

Obviously the allosteric regulation of the virus-induced enzyme—if regulated at all—is quite different from the regulation of the cellular reductase. We hope that further studies on the herpesvirus-induced ribonucleotide reductase will give valuable information on the control and specific requirements of herpesvirus DNA synthesis.

ACKNOWLEDGMENTS

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