Allosteric Regulation of Vaccinia Virus Ribonucleotide Reductase, Analyzed by Simultaneous Monitoring of Its Four Activities*

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As determined by simultaneous monitoring of its four activities, vaccinia virus-coded ribonucleoside diphosphate (rNDP) reductase shows responses to individual nucleoside triphosphate effectors—ATP, dATP, dGTP, and dTTP—similar to those previously reported for rNDP reductase of mouse, which the viral enzyme closely resembles. This investigation uses the vaccinia enzyme as a readily available and convenient model for understanding the cellular enzyme. As previously reported for T4 phage aerobic rNDP reductase, we found the relative activities of ADP, CDP, GDP, and UDP reductase to be reasonably close to the proportions of the four deoxyribonucleotides in the vaccinia virus genome, but only when the four substrates and the four allosteric effectors were all provided at their approximate intracellular concentrations. GDP reductase levels were somewhat higher, proportionately, than the representation of dGMP in vaccinia virus DNA. To understand this behavior and also to evaluate possible relationships between ribonucleotide reductase control and the very low dGTP pools seen in eukaryotic cells, we carried out substrate saturation experiments with a “bioproportional” mixture containing the four rNDP substrates at their relative in vivo concentrations as determined from rNDP pool measurements. Reduction of the two purine substrates was inhibited at high concentrations of this mixture, and data suggest that ADP acts as a specific inhibitor of its own reduction and that of GDP. Use of the four-substrate assay revealed also that a mixture of vaccinia virus R1 protein and mouse R2 protein is catalytically active, making this the first reported chimeric rNDP reductase to show biological activity.

The allosteric regulation of ribonucleotide reductase (RNR) by individual nucleoside triphosphates is well characterized for several forms of the enzyme (1, 2). The three classes of RNR, although quite different in structure (3), have similar allosteric behavior in terms of the effects of modifiers upon substrate specificity. In all classes, ATP and dATP (when it is not inhibitory) activate reduction of the cytidine and uridine nucleotides. Reduction of adenosine and guanosine nucleotides is stimulated by dGTP and dTTP, respectively. These effects are mediated through binding of nucleotides to two different allosteric sites on the dimeric R1 (large) protein: the activity sites, which bind with relatively low affinity ATP (general activator) or dATP (general inhibitor), and the specificity sites, which bind with higher affinity ATP, dATP, dGTP, or dCTP, with the binding of each ligand activating the inhibition of some substrates and inhibiting that of others.

However, due primarily to limitations of traditional RNR assay methods, only the isolated effects of individual allosteric effectors have been determined, in kinetic experiments involving single substrates. We have developed an assay procedure (4) that permits simultaneous monitoring of the four rNR activities. With this procedure, the effects of more complex nucleotide environments on substrate turnover rates can be examined. In addition, other factors that influence relative turnover rates, such as protein-protein interactions, can be evaluated by this technique.

Because of the ease with which it can be purified in large quantities as a recombinant enzyme, we are using the class I RNR encoded by vaccinia virus as a model for the closely related mammalian cell enzymes. The two viral RNR genes express proteins similar in size to mammalian R1 (large) and R2 (small) RNR proteins, and the molecular weight of the holoenzyme suggests the αβ2 tetrameric structure characteristic of the cellular enzymes. Also, like the cellular RNRs, the viral enzyme reduces ribonucleoside diphosphates to the corresponding deoxyribonucleotides, via a free radical mechanism that involves a tyrosyl residue on the R2 subunit (5), with catalytic and allosteric sites located on the R1 subunit. The vaccinia R1 and R2 proteins share about 80% amino acid sequence identity with corresponding mammalian RNR proteins (6, 7). Preliminary data, obtained when the vaccinia enzyme was first described in our laboratory (8), indicate that the viral enzyme is regulated similarly to the mammalian enzyme. This study explored that regulation in more detail, using the four-substrate assay.

MATERIALS AND METHODS

Overexpression and Purification of Recombinant Subunits—Both subunits of vaccinia ribonucleotide reductase were previously cloned into isopropyl-1-thio-β-D-galactopyranoside-inducible pET expression vectors and were overexpressed as described (5, 9). The mouse R2 expression system (10) was a gift from the laboratory of Lars Thelander (Umeå University, Umeå, Sweden). Overexpression of the mouse R2 was carried out according to the procedure used to express the vaccinia R2. The mouse R2 clone contains two selectable markers encoding drug resistance gene products, and therefore, the addition of 25 μg/ml chloramphenicol was required in conjunction with 100 μg/ml ampicillin. The purification procedure for the vaccinia R2 subunit was essentially as reported (5), except for the incorporation of an additional radical reactivation step, described in the accompanying paper (11). Mouse R2 protein was purified by the same procedure used to purify the vaccinia R2 (5). Purification of the vaccinia R1 protein was performed...
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RESULTS

Ribonucleotide Reductase Activities in the Presence of the General Allosteric Effector ATP—Fig. 1 demonstrates the application of the four-substrate RNR assay to the purified vaccinia virus enzyme. In this experiment, ATP (2.5 mM) was added as the sole allosteric modifier. Under these conditions, CDP and GDP were the principal substrates reduced. Reduction of UDP was low or negligible in this and all other assays performed. Mammalian cells have been shown to derive most of their DNA thymidylate residues from deoxyxystidine nucleotides, via the dCMP deaminase reaction, rather than through the reduction of uridine nucleotides (14), and these data suggest that the same situation holds true for vaccinia virus-infected cells. In this experiment, the total RNR activity, summed for the four substrates, was 77 nmol/min/mg of R1 protein. This value corresponds to a turnover number of 12.4 min⁻¹, comparable to values reported for the recombinant mouse RNR (10, 15).

Regulation of the Vaccinia RNR by Nucleoside Triphosphate Effectors—Most of our present understanding of the allosteric regulation of mammalian rNDP reductase is based upon analysis of the effects of one or two modifiers—typically, an activity site effector (dATP or ATP), in the presence or absence of a specificity site ligand (ATP, dATP, dGTP, or dTTP)—upon the reduction of single substrates. To compare the vaccinia virus rNDP reductase with its mammalian counterpart, we determined the effect of each of the four dNTPs upon the four activities of the virus enzyme in the presence of ATP, in comparison with an assay carried out in the presence of ATP alone. As shown in Fig. 2, the viral enzyme behaves virtually identically to the reported behavior of mammalian rNDP reductases.

dCTP has no significant effect on any of the activities, whereas dTTP activates GDP reduction and inhibits reduction of ADP and CDP. dGTP activates reduction of ADP and inhibits CDP reduction, whereas dATP, at the concentration used, inhibits all four reactions. In this experiment, the percentage of inhibition of the reduction of each substrate by dATP (relative to the control in Fig. 2A) was 36% for ADP, 38% for CDP, 46% for GDP, and 40% for UDP. These effects are summarized in Table I. Other experiments (not presented) showed CDP reduction to be stimulated by dATP, when added in the absence of ATP, consistent with the action of dATP as a positive effector for CDP reduction when bound at the specificity site. At higher concentrations, the inhibitory effect of dATP (in the presence of ATP) increased, as shown in Fig. 3. Within the likely physiological dATP concentration range (5–15 μM), the activity (sum of the four activities) was inhibited by 25–50%, values comparable to those reported for single substrates with the mammalian enzymes. These data confirm the appropriateness of using vaccinia RNDP reductase as a model for understanding cellular eukaryotic forms.

rNDP Concentrations in Vaccinia Virus-infected BSC₄₀ Cells—Our application of the four-substrate assay to T4 phage-coded aerobic ribonucleotide reductase indicated that intracellular ribonucleoside diphosphate concentrations constitute a significant regulatory factor. The relative rates of the four reactions corresponded closely to the nucleotide composition of T4 DNA, but only under conditions where all four nucleoside triphosphate effectors and all four nucleoside diphosphate substrates were present. These effects were also estimated from pool size measurements. In determining whether the vaccinia rNDP reductase behaves similarly, it was first necessary to measure ribonucleoside diphosphate pool sizes in vaccinia virus-infected cells. These values, determined by HPLC measurements of virus-infected cell extracts as described under "Materials and Methods," are as described in the accompanying paper (11).
presented in Table II. The approximate concentrations reported fall within the ranges of values compiled by Traut (16) for uninfected mammalian cell cultures. It should be observed that the HPLC peaks from which these pool sizes were determined almost certainly include deoxyribonucleoside diphosphates, as well as the ribonucleotides. However, the data compiled by Traut (16) indicate that in mammalian cells, the rNDP pools exceed those of dNDPs by 100–1000-fold. Therefore, we assumed the contribution to peak area by the dNDPs to be negligible.

To estimate the in vivo molar concentration of each rNDP from the pool size values in pmol/10⁶ cells, we used 2 picoliters per cell, a mid-range value for the compiled volumes of cultured mammalian cells, as reported by Hauschka (13). These estimated concentrations, listed in Table II, are quite close to average concentrations of rNDPs in a range of mammalian cells (16).

Effects of Natural Nucleotide Pool Asymmetries upon dNDP Product Profiles—As noted earlier, the T4 aerobic rNDP reductase in vitro reduces the four substrates at rates corresponding to the nucleotide composition of T4 DNA, but only under conditions where both substrates and allosteric effectors are present at their approximate intracellular concentrations. In mammalian cells, the ribonucleoside diphosphate pools are more strikingly asymmetric than in T4 phage-infected *Escherichia coli*, with more than an order of magnitude variation between the most (ADP) and least (CDP) abundant (Table II and Ref. 16), whereas the corresponding asymmetry in the bacterial system is less than 3-fold (17). Table III summarizes results of assays carried out under different conditions of substrate and

![FIG. 2](image-url) Regulation of vaccinia virus ribonucleotide reductase activities by individual dNTPs. All reaction mixtures contained ATP at 2.5 mM and all four rNDP substrates at 0.15 mM each. Individual dNTP effectors were added to a final concentration of 10 μM. Reaction mixtures were incubated at 37 °C for 6 min.

![FIG. 3](image-url) Inhibition of vaccinia virus RNR by dATP. This figure shows the total RNR activity (sum of the four activities) remaining after addition of dATP to the reaction mixture. The decrease in activity was calculated by comparing the total amount of rNDP reduced in the presence of 2.5 mM ATP with the amount reduced when the reaction mixture contained 2.5 mM ATP plus dATP at the indicated concentration.

| dNTP (10 μM) | Activates reduction of | Inhibits reduction of |
|--------------|------------------------|----------------------|
| dCTP         | No effect              | No effect            |
| dTTP         | GDP                    | CDP and ADP          |
| dGTP         | ADP                    | CDP                  |
| dATP         | ADP, CDP, GDP, and UDP |                      |

![TABLE I](image-url) Regulation of vaccinia virus ribonucleotide reductase activities by single dNTPs

These results represent changes that occur in rNDP turnover rates relative to the rates observed when the enzyme is stimulated by ATP only. Activation of CDP reduction by dATP was evident only when ATP was not added to the reaction mixture. When dATP was added in conjunction with ATP (0.5–2.5 mM), all activities were inhibited relative to a control reaction containing only ATP.

![TABLE II](image-url) Ribonucleoside diphosphate concentrations in vaccinia virus-infected BSC40 cells

*Mean ± (range/2) for two independent determinations.

Volume of a vaccinia-infected BSC40 cell was estimated at 2 picoliters, in the middle of the range of values reported for cultured mammalian cells.
Ribonucleotide Reductase Control with a Four-substrate Assay

Effects of variations in substrate and effector concentrations upon specificity of vaccinia ribonucleotide reductase in the four-substrate assay

All reaction mixtures contained ATP at 2.5 mM. Equimolar substrates were present at 0.15 mM each. In vivo substrate levels were as follows: CDP, 50 μM; UDP, 250 μM; ADP, 600 μM; and GDP, 100 μM. In vivo allosteric effector concentrations were 15, 10, and 5 μM dATP, dTTP, and dGTP, respectively. Reaction mixtures were incubated for 5 min, an interval within which all reactions were linear with respect to time.

Table III

| Assay conditions | Percentage of total product formed as | dCDP | dUDP | dADP | dGDP |
|------------------|-------------------------------------|------|------|------|------|
| ATP only (equimolar substrates) | 76 | 3 | 2 | 19 |
| ATP only (in vivo substrates) | 49 | 15 | 8 | 28 |
| In vivo effectors (equimolar substrates) | 60 | 3 | 15 | 22 |
| In vivo effectors (in vivo substrates) | 34 | 12 | 29 | 26 |

Note: The percentages represent the relative concentrations of the four rNDPs, with UDP present at a ratio of 12:1:2:5, respectively (33% of vaccinia genomic composition is thymine).

The small but significant decrease in GDP reduction at the highest rNDP concentrations could mean that true intracellular concentrations sensed by RNR are somewhat higher than those estimated in the experiment of Table II. Thus, in vivo flux rates for vaccinia virus rNDP reductase may conform even more closely to the nucleotide composition of vaccinia DNA than does the enzyme in the "in vitro" experiment of Table III.

Although limited, the data suggest that inhibition of ADP reached or approached at the highest concentrations tested. However, ADP and GDP both showed inhibition at the highest concentrations tested. The effect of increased concentration upon ADP reduction was particularly striking, with the highest rate of ADP reduction seen at the lowest concentration.

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Effects of Proportional Variations in rNDP Substrate Concentrations—The data of Table III show one significant discrepancy between rNDP substrates. Under physiological conditions, approximately equal proportions of dCDP, dUDP, dADP, and dGDP were present in the genome of the vaccinia virus. However, of greater interest is the mechanism by which purine rNDP reduction, particularly that of ADP, evidently becomes inhibited at higher concentrations. Note that because of its high representation among the rNDPs, ADP reduction rates are quite high at the lowest levels tested, because it is probably close to saturating the enzyme under these conditions. This factor—the abundance of ADP—could make it advantageous for the ADP reduction flux to be limited at the higher concentrations needed for the enzyme to act efficiently upon the other three rNDPs. In particular, this could be seen as a factor limiting the accumulation of dATP, which could otherwise block DNA replication through activity site binding to rNDP reductase, with consequent depletions of the pools of dCTP, dGTP, and dTTP.

But is ADP the actual inhibitory species? In principle, inhibition could be brought about by the product, dADP, because deoxyribonucleoside diphosphates have been reported to act as rNDP reductase regulators under some conditions. This seems unlikely, because reaction rates were constant over the interval of each experiment (data not shown). Had dADP accumulation been responsible for the inhibition, then its progressive accumulation would be expected to progressively slow down the rates of ADP and GDP reduction. Moreover, the smallest amounts of dADP were formed in the reaction mixtures showing the greatest inhibition.

Alternatively, if nucleoside diphosphate kinase were present in the reaction mixtures, the dADP formed could be converted to dATP. This possibility can be ruled out, first, because dATP accumulation would inhibit all four activities, not just those on purine substrates, and second, because our vaccinia R1 and R2 preparations have no detectable NDP kinase activity (which would have been detectable in our assay system). This can be seen from the data of Fig. 2, where the amount of each dNTP tested as an allosteric modifier was always close to the concentration added to the reaction mixture at time zero. Had NDP kinase been present, the amounts of dNTPs would have increased over the time of incubation. The ability to detect a number of potential containing enzyme activities represents an additional strength of the four-substrate assay procedure.

Although limited, the data suggest that inhibition of ADP...
and GDP reduction at high rNDP concentrations is brought about by ADP itself. This was tested in the experiment shown in Fig. 5, in which the concentration of ADP alone was varied in the presence of constant amounts of CDP (50 μM) and GDP (100 μM). CDP reduction was unaffected over the ADP concentration range tested (50–600 μM), whereas the reduction of both purine nucleotide substrates was substantially inhibited. We have not yet asked directly whether GDP is inhibitory, but if both ADP and GDP are inhibitors, the data shown in Fig. 4 suggest that the effect of ADP is considerably more pronounced.

Activity of a Chimeric RNR Holoenzyme—From the above, it is clear that an important goal is to learn whether rNDP reductase inhibition by ADP is a general regulatory feature of this enzyme, by carrying out kinetic experiments of the kind described here with a mammalian form of the enzyme. As noted earlier, the vaccinia virus enzyme represents a good model for the mammalian RNR. We had an opportunity to test this concept further, because of the availability of mouse R2 protein, purified as a recombinant protein from an overexpressing clone furnished by Dr. Lars Thelander (Umeå University). It was a simple matter to ask whether this protein could function effectively with the vaccinia virus R1 protein in a four-substrate assay. As shown in Fig. 6, the answer was yes. Under the reaction conditions tested (2.5 mM ATP, no dNTP effectors, equimolar rNDP mix at 0.15 mM each), the reduction of UDP and GDP was negligible, but the activities on ADP and CDP were considerable, with the specific activity from the reduction of all four substrates amounting to 48 nmol dNDP-min⁻¹·mg R1⁻¹, which corresponds to a turnover number of 7.6 min⁻¹. This is similar to the turnover number determined for the native vaccinia R1-R2 complex (12.4 min⁻¹, determined in the experiment shown in Fig. 1).

In evaluating this experiment, we must consider the possibility that the activity results from E. coli R1 and R2 proteins, which are present as minor contaminants in our recombinant vaccinia and mouse protein preparations. Three observations rule against this possibility. First, in the experiment shown in Fig. 6, neither subunit had detectable activity when tested alone (data not shown). Second, the dATP-Sepharose column protocol used to purify vaccinia R1 protein is specifically designed to remove any E. coli R1. Vaccinia R1 requires 75 mM ATP for elution from the column, whereas E. coli R1 is efficiently eluted at 5 mM ATP; therefore, our procedure involved a 5 mM ATP elution step before desorption of the vaccinia R1 at 75 mM ATP elution. Third, in experiments not shown, we have run a four-substrate assay on recombinant E. coli R1-R2 complex under conditions identical to those of Fig. 6. The product profile was quite different, with the order of activities for the E. coli enzyme being A > G > C > U. By contrast, the chimeric enzyme depicted in Fig. 6 showed relative activities C > A > U > G.

**DISCUSSION**

Allosteric Regulation of Vaccinia Virus Ribonucleotide Reductase—The principal aim of this study was to apply the four-substrate ribonucleotide reductase assay to an understanding of the metabolic regulation of a eukaryotic form of the enzyme, using the vaccinia virus RNR as a convenient model for the mammalian cell enzyme. The viral enzyme behaved identically to mammalian RNRs, as shown previously in single-substrate assays (1, 2, 20), in its response to individual dNTPs. As noted in Table I, dCTP had no discernible effect on any of the four reactions, whereas dATP inhibited all four to about the same extent and at concentrations shown to have similar effects on the mammalian enzyme. For example, the calf thymus enzyme was earlier found to have 43% of the control activity for CDP reduction at 10 μM dATP (20), a value very close to that suggested from Fig. 3 for the four-substrate assay of the vaccinia enzyme. The specific stimulatory and inhibitory effects of dGTP and dTTP are also identical to those noted previously for the mammalian RNRs. Presumably, all of these effects involve binding to activity and specificity sites, as has previously been described for the mammalian enzyme (1, 2). The total activity (sum of the four individual activities) was highest in the pres-
ence of ATP alone, the general allosteric activator. The turnover number determined, 12.4 min⁻¹, was somewhat lower than values determined from single-substrate assays for either the recombinant vaccinia enzyme (18 min⁻¹) (9) or the recombinant mouse enzyme (24 min⁻¹) (15). However, data from our laboratory (21) indicate that the vaccinia RNR is present in significant excess over its need to provide precursors for viral genome replication at observed rates, even when assayed with the nonphysiological electron donor dithiothreitol.

However, the individual effects upon enzyme specificity of a partial recreation of intracellular conditions will be much harder to discern. The data of Table III clearly establish the importance of running reactions in the presence of physiological concentrations of both nucleoside diphosphate substrates and nucleoside triphosphate effectors. The individual regulatory effects involved include an undeniably complex mixture of the following: allosteric interactions of ATP and dNTPs at activity and specificity sites, competition among the four rNDP substrates for binding to catalytic sites, product inhibition by dNDPs, possible interactions of both ribo- and deoxyribonucleoside diphosphates with activity and specificity sites (19, 22, 23), and (possibly related to those interactions) the apparent inhibition of pyrimidine rNDP reduction by ADP. Previous studies have indicated that mammalian ribonucleotide reductase activities are influenced by both rNDP substrates and dNDP products (19, 22, 23). In some, but not all, of these earlier studies, inhibition by rNDPs was competitive, as expected if alternative substrates are competing for binding to catalytic sites. However, in some studies, the inhibitory effects of alternative rNDP substrates were found to be noncompetitive (22, 23), suggesting that binding occurs at a location other than the catalytic site. One of these studies (22) was carried out with the herpes simplex type 1 rNDP reductase, an enzyme that is distinctive in being largely free of allosteric control mediated by nucleoside triphosphates. The data suggested that regulation with this enzyme is accomplished by a mixture of competitive binding of substrates and inhibition by dNDP products, both effects occurring at catalytic sites.

From existing data (19, 22, 23), it is evident that deoxyribonucleoside diphosphates function not only by product inhibi-

tion, but similarly to their triphosphate counterparts, presumably by interaction with allosteric sites. Data on dNDP pool sizes, necessary to establish the biological significance of these effects, are sparse. However, those data that do exist (16) suggest that under most circumstances, dNDP levels are far lower than those shown to have significant allosteric effects in vitro. The pool sizes of ribonucleoside diphosphates are much higher, but the complexity of considering catalysis, binding to catalytic sites, competition with alternative substrates for binding, and possible interaction with allosteric or still undefined sites indicates that physical studies of nucleotide binding will be needed to understand the regulatory effects of rNDPs.

Substrate Inhibition of Ribonucleotide Reductase Activity—

However, the most interesting result of our bioproportional rNDP mixture, varying the concentrations of all four substrates in proportions that represented the relative pool sizes of these four nucleotides. This led in turn to our finding that GDP reduction rates decrease at higher substrate concentrations, suggesting that relatively small adjustments in substrate concentrations would allow us to approach even more closely a product profile that truly represents vaccinia virus DNA base composition.

Our observations led us to carry out substrate saturation experiments with a bioproportional rNDP mixture, varying the concentrations of all four substrates in proportions that represented the relative pool sizes of these four nucleotides. This led in turn to our finding that GDP reduction rates decrease at higher substrate concentrations, suggesting that relatively small adjustments in substrate concentrations would allow us to approach even more closely a product profile that truly represents vaccinia virus DNA base composition.
at the expense of ADP reduction. Therefore, as the concentration of ADP is increased, the rate of ADP reduction should decrease relative to the reduction of the other substrates. This hypothesis is also consistent with the inhibition of GDP reduction observed at higher levels of the bioproportional mixture. Testing the hypothesis, however, will require physical studies of ADP binding to the R1 protein.

Is the inhibition by ADP physiologically significant? Because the effect is seen in vitro at nucleotide concentrations corresponding to in vivo levels, that seems a reasonable supposition. Possibly this could represent a mechanism to prevent the accumulation under certain circumstances of dATP, which has been shown at low concentrations in vitro to stimulate activations of proteases in a cascade of events leading to apoptosis (24) and which is also a potent inhibitor of DNA replication through its effect on ribonucleotide reductase. Inhibition by ADP could also help explain the phenomenon explored in the companion paper, namely, the specific inhibition of ADP reduction in vivo by hydroxyurea (11). If hydroxyurea inhibition of RNR causes all four rNDPs to accumulate in the short term, then the accumulation of ADP, the most abundant rNDP, could lead to more specific inhibition of ADP reductase in the longer term, with a consequent specific depletion of dATP pools.

The Vaccinia-Mouse RNR Chimera—Although not related to the central theme of this investigation, it was interesting to learn that a hybrid form of rNDP reductase, containing vaccinia R1 and mouse R2, had enzymatic activity comparable to that of wild-type forms of the enzyme. Previous attempts to generate active chimeras of the T4 and E. coli RNRs had revealed no activity (25).

When one considers what is known about the interactions between R1 and R2, the activity of a mouse-vaccinia chimera may not be so surprising. The large and small subunits of the class I RNRs interact primarily through highly specific contacts formed between the R2 carboxyl terminus and its corresponding binding site located on the R1 protein. The R2 C-terminal sequences are not conserved but are highly species-specific. But these sequences in the mouse and vaccinia proteins are closely related, as shown by the following comparison of the 10 C-terminal residues of mouse and vaccinia R2 proteins.

Mouse: ENSFTLDADF-coo
Vaccinia: DNHFLDVDF-coo

The underlined residues represent amino acids in the vaccinia R2 protein that differ from those of the mouse (which is identical to the human sequence). All of the differences except one represent conservative substitutions. The activity of the chimeric enzyme suggests that the vaccinia enzyme is sufficiently closely related to mammalian RNR to serve as a good model system. In addition, the activity of a mouse R1-virus R2 chimera in vitro raises the interesting question of whether such enzymes are formed in vivo, in vaccinia virus-infected cells. Although we might expect the viral subunits to interact more strongly with each other than with corresponding host cell proteins, such chimeras might form in infection with viral mutants lacking either R1 or R2 proteins. Elsewhere, we have discussed the question of whether such a complex might form in vivo, in infection by a vaccinia virus mutant with a site-specific deletion of the R1 gene (26). With the availability of recombinant mouse R1 (15), the stage is set for asking whether this chimeric enzyme actually can form in vitro.

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