Active Site of Ribonucleoside Diphosphate Reductase from 
Escherichia coli

INACTIVATION OF THE ENZYME BY 2'-SUBSTITUTED RIBONUCLEOSIDE DIPHOSPHATES*

(Received for publication, September 5, 1975)

LARS THELANDER AND BENGT LARSSON
From the Medical Nobel Institute, Department of Biochemistry, Karolinska Institute, Stockholm, Sweden

JOHN HOBBS AND FRITZ ECKSTEIN
From the Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, Göttingen, Germany

Ribonucleoside diphosphate reductase is an allosteric enzyme consisting of two nonidentical subunits, proteins B1 and B2. B1 contains dithiols which participate in the oxidation-reduction reactions of electron transport, while B2 contains a free radical essential for activity. Ribonucleoside diphosphates are bound to B1 but not to B2.

Addition of 2'-deoxy-2'-chloro ribonucleoside diphosphates to ribonucleotide reductase irreversibly inactivates B1 without affecting B2. The reaction is specific since (a) it requires the presence of active B2, (b) it is controlled by allosteric effectors, (c) B1 is protected against inactivation by the normal substrates, and (d) the chloro-substituted nucleoside monophosphates have no effect. The inactivation of B1 is caused by a modification of the oxidation-reduction dithiols. The chloro derivatives decompose into free base, chloride ion, and 2-deoxyribose 5-diphosphate as a consequence of reaction with the enzyme.

2'-Deoxy-2'-azido ribonucleoside diphosphates cause an irreversible inactivation of B2 without affecting B1. The reaction is specific by the above criteria, indicating that the azido derivatives also bind to the active site of the enzyme. On reduction by ribonucleotide reductase, the azido derivatives function as radical scavengers and selectively destroy the free radical of B2, indicating that this radical participates in ribonucleotide reduction directly.

On the basis of these results, a model of the active site of ribonucleotide reductase is proposed in which the site is formed from both B1 and B2. In the site the electron-donating oxidation-reduction active dithiols of B1 are in close contact with the free radical of B2.

The azido derivative also inactivates the adenosylcobalamin-dependent ribonucleoside triphosphate reductase from Lactobacillus leichmannii and a ribonucleotide reductase preparation from calf thymus, indicating a general involvement of free radical intermediates in enzyme-catalyzed ribonucleotide reduction.

---

*This investigation was supported by grants from The Swedish Medical Research Council, Magnus Bergvalls Stiftelse, The Medical Faculty of the Karolinska Institutet, The Deutsche Forschungsgemeinschaft, and a short term fellowship from the European Microbiology Association.
The active dithiols, capable of interacting with thioredoxin, and contains binding sites both for the ribonucleoside diphosphate substrates and for the nucleoside triphosphate effectors. Protein B2 has a molecular weight of 78,000 and contains bound non-heme iron and an organic free radical essential for activity (3, 5). No binding of substrates or effectors to B2 can be demonstrated.

In this paper the interaction between ribonucleotide reductase and some 2'-modified ribonucleoside diphosphates is studied. The nucleotides were of two types; 2'-deoxy-2'-chloronucleoside 5'-diphosphates and 2'-deoxy-2'-azidonucleoside 5'-diphosphates (Fig. 2). The results indicate that both B1 and B2 contribute to the active site of the enzyme and that the radical present in B2 directly participates in the catalytic process together with the oxidation-reduction active dithiols of B1.

**EXPERIMENTAL PROCEDURE**

**Materials**

Proteins B1 and B2 were described as earlier (3). Ribonucleotide reductase from *Lactobacillus leichmannii* was prepared according to Chen et al. (6) and had a specific activity of 7 μmol of CTP reduced/hour/mg of protein at 37°C. The preparation of ribonucleotide reductase from calf thymus will be described elsewhere. The 5'-diphosphates of 2'-deoxy-2'-chlorocytidine-5'-triphosphate and 2'-deoxy-2'-azidouridine-5'-triphosphate were synthesized as described by Hobbs et al. (7). [α-32P]CTP was synthesized by heating 0.2-μmole amounts of HPOCl (7). [35Cl]UDP was synthesized by heating 0.2-μmole amounts of sodium dithiothreitol with LiCl in dimethylformamide (8) followed by phosphorylation (7). [35Cl]UDP was prepared from 35ClDP by the exchange reaction with bisulfite (9), and [35Cl]UDP was added as substrate. Calf thymus ribonucleotide reductase was assayed as described by Larsson (16) with the modification that 1.3 nmol of CclDP (75 nmol) was added to the cuvettes except for the control one before addition of the sample to prevent CclDP from interfering with the assay.

Protection by CDP against CclDP-inactivation of B1—Into four microcuvettes 50 μl of ATP assay mixture, 0.15 nmol of B2, 25 to 750 nmol of CDP, and 5 nmol of CclDP were pipetted, and the final volume was adjusted with water to 126 μl. As a control, an identical cuvette was prepared containing no CclDP but 75 nmol of CDP. After mixing, the reaction was started by the addition of 0.016 nmol of B1 in 3 μl of 0.05 M Tris-Cl, pH 7.6. Initial reaction rates were calculated as described earlier (15).

Titration of Protein B1 with CclDP—A series of tubes was prepared on ice, each containing 0.27 nmol of B1 and 0.22 nmol of B2 in 23 μl of 0.05 M Tris-Cl, pH 7.6, 0.011 M MgCl2, 5 × 10^-4 M dTTP. One tube was kept as a control, and to the rest 0.11 to 4.7 nmol of CclDP in 0.05 M Tris-Cl, pH 7.6, were added to give a final volume of 25 μl. After 45 min of incubation at 25°C, 2-μl aliquots were taken for assay of B1 activity using a dTTP assay mixture. CDP was added to all the cuvettes except for the control one before addition of the sample to prevent CclDP from interfering with the assay.

Protection by CDP against CclDP-inactivation of B2—Into four microcuvettes 50 μl of ATP assay mixture, 0.08 nmol of B2, 25 to 750 nmol of CDP, and 5 nmol of CclDP were pipetted, and the final volume was adjusted with water to 126 μl. As a control, an identical cuvette was prepared containing 75 nmol of CDP but no CclDP. The reaction was started by the addition of 0.015 nmol of B2 in 6 μl of 0.05 M Tris-Cl, pH 7.6. Activity was measured as described earlier (15).

Identification of Reaction Products of 2'-Substituted Ribonucleoside Diphosphate Analogues—Aliquots of the incubation mixtures were deproteinized by adding HClO4 to a final concentration of 0.7 M or by mixing 2 volumes of 90% ethanol with 1 volume of sample. Prior to chromatography, the HClO4 was neutralized with KOH using phenol red as an indicator, and the KClO4, precipitate was removed by centrifugation. The deproteinized samples were chromatographed on Whatman no. 3MM paper or on polyethyleneimine plates together with appropriate markers. All media used for paper chromatography were as described by Harbers (17).

[35Cl]Cytosine was detected in incubation mixtures by paper chromatography in 99% water-saturated 1-butanol/1% ammonia (CDCl and CcIDP remain at the starting point and cytosine moves ahead of phenol red) or in 65% isopropanol/35% 5.7 M HCl (cytosine and cytidine move ahead of 2'-chlorocytidine and 2'-deoxycytidine), followed by chromatography in a borate-containing medium (2), where cytosine moves with the solvent front and cytidine is retarded.

[35Cl]Uracil was identified by paper chromatography in isobutyric acid/ammonia/water (60/1/33) (Table I, A).

Free [35Cl] was determined by paper chromatography in 95% ethanol/1 M ammonium acetate, pH 5.0, containing 0.01% EDTA (70/30) (Table I, B).

Chromatography on polyethyleneimine plates was performed in a medium containing 60 g of ammonium sulfate dissolved in 100 ml of 0.1 M potassium phosphate buffer, pH 7.0, plus 2 ml of 1-propanol (Table I, C), and (b) 1 M LiCl (Table I, D). Before the LiCl was added, the plates were run for 2 cm in water.

Radioactive spots were localized by cutting the papers or plates into pieces of equal size, adding scintillation fluid containing solvane/
Catalytic amounts of B2 were sufficient to inactivate at least a 10-fold molar excess of B1, and only B1 was inactivated in a B1/B2 mixture, since after complete inactivation of B1 fully active B2 could be isolated from the incubation mixture by hydroxyapatite chromatography (15).

CDP Protects Protein B1 against CclDP

Protection of B1 against inactivation by CclDP was observed when increasing amounts of CDP were added to B1 assay mixtures together with a fixed amount of CclDP and the initial reaction rates were compared (see "Experimental Procedure"). The reaction rates decreased with time and finally stopped completely. This occurred after only a few minutes in the cuvette containing the lowest CDP concentration, but required about 10 min in the cuvette containing the highest CDP concentration. At these times most of the added CDP was not reduced.

The results indicate competition between CclDP and CDP for the same site on the enzyme. Addition of CclDP or 2'-deoxy-2'-chlorocytidine to a B1/B2 mixture gave no inactivation of B1, indicating that specific binding of the chloro analog to the active site of the enzyme was necessary for inactivation.

Influence of Effectors on Inactivation by CclDP

The inactivation of protein B1 by CclDP was much faster in the presence of a positive effector than in the presence of a negative one, as shown in Table II, where the effects of ATP and dATP are compared. Addition of dATP and ATP together (to reverse the inhibition by dATP) again made protein B1 more sensitive to inactivation by CclDP (Table II, 4). Inactivation in the presence of dGTP was slower than in the presence of ATP or dTTP, but faster than with dATP (data not shown). The results indicate that the interaction of CclDP with ribonucleotide reductase is controlled by allosteric effectors in the same way as the binding of normal ribonucleoside diphosphate substrates (4), and that, therefore, the interaction occurs at the substrate binding site of the enzyme.

Reaction Products of the Chloro-substituted Analog

UclDP containing [32P]Cl, CclDP, and CDP labeled with tritium in the base and [α-32P]CDP were used to investigate the nature of the end products formed during incubation of the analogs with ribonucleoside reductase. Both free base (uracil or cytosine) and chloride ions were released from the chloro-substituted analogs during the reaction and could be isolated from the incubation mixtures and identified by paper or thin layer chromatography (see "Experimental Procedure"). The remaining part of the analog molecules behaved like 2-deoxyribose 5-diphosphate on paper electrophoresis or polyethyleneimine thin layer chromatography. No release of chloride ions or base occurred on incubation with protein B1 in the absence of B2. Radioactivity was never found bound to protein after removal of low molecular weight components from the incubation mixtures by chromatography on Sephadex G-50 columns, regardless of the nature of the radioactive label.

Kinetics of UclDP Inactivation of B1

Limiting Amounts of UclDP—Fig. 3 shows the results obtained when 3 mol of [32P]UclDP/mol of B1 were added to an equimolar mixture of B1 and B2 together with Mg2+ and dTTP. Before and after the addition of UclDP, samples were
Influence of the positive effector ATP and the negative effector dATP on CDP-inactivation of protein B1

Table II

<table>
<thead>
<tr>
<th>No.</th>
<th>Conditions</th>
<th>Remaining B1 activity after 30 min at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>CDP + ATP</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>CDP + dATP</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>CDP + dATP + ATP</td>
<td>50</td>
</tr>
</tbody>
</table>

Excess CDP—This experiment was performed as above except that 11.0 mol of [3Cl]CDP were added/mol of B1 (Fig. 4). There was again a fast inactivation of B1 coupled to a release of chloride ion and a disappearance of titrable sulfhydryl groups. However, there was also a slow release of chloride ion even after protein B1 had been inactivated completely, until all CDP had disappeared. The reason for this is not known. As before, the absorbance at 320 nm increased slowly during the whole period. Similar results were obtained with CDP labeled with tritium in the base, indicating that the base and the Cl⁻ were released simultaneously.

Correlation between Inactivation of B1 and CDP-mediated modification of the Oxidation-Reduction Active Dithiols

Adding an excess CDP (but not CDP) to a B1/B2 mixture resulted in the loss of about 6 titrable sulfhydryl groups/mol of B1 at complete inactivation (Fig. 4). A similar loss of titrable sulfhydryl groups occurred on addition of the normal substrate CDP, but without inactivation (2). In the latter case, the sulfhydryl groups were easily regenerated by addition of dithiothreitol or reduced thioredoxin, while neither sulfhydryl nor B1 activity could be regenerated after inactivation by CDP. The failure of dithiothreitol or reduced thioredoxin, while neither sulfhydryl nor B1 activity could be regenerated after inactivation by CDP.

What changes of the active dithiols of B1 occurred during inactivation by CDP? In Table III, the number of cysteic acid residues recovered after performic acid oxidation and acid hydrolysis of a CDP-inactivated B1-B2 complex is compared to the number of such residues recovered after an identical
Cys(OH) per
residue
Cys(OH) per
residue
mol/mol
CDP (control)
0.130
0.126
CeDP
0.099
0.091

Nature of Absorbance Peak at 320 nm

Whenever a chloro-substituted analog was added to a Bl:B2 mixture, a slow increase in the absorbance at 320 nm was observed (Figs. 3 and 4). After chromatography on Sephadex G-50, most if not all of this absorbance was excluded from the column. When Bl and B2 were separated on dATP-Sepharose (3) after inactivation, the 320 nm absorbance was recovered with Bl. Figs. 3 and 4 show that the absorbance increased with incubation time and continued after the inactivation of Bl had reached a plateau value. After about 40 min of incubation of Bl and B2 with an excess of chloro analog, most of the 320 nm absorbance had developed, and the spectrum of protein Bl looked as shown in Fig. 5.

The A320 peak did not disappear when Bl inactivated by CeDP was passed through a Sephadex G-25 column equilibrated with 6 M guanidine hydrochloride or after precipitation of the protein with trichloroacetic acid and solution of the

Cysteic acid residues recovered after perimetric acid oxidation and hydrolysis of a Bl:B2 mixture incubated with CeDP as compared to a control incubated with CDP

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Cys(OH) per aspartic acid residue</th>
<th>Cys(OH) per glutamic acid residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDP (control)</td>
<td>0.130</td>
<td>0.126</td>
</tr>
<tr>
<td>CeDP</td>
<td>0.099</td>
<td>0.091</td>
</tr>
</tbody>
</table>

CeDP Inactivates Protein B2 in Presence of Bl

Addition of increasing amounts of CeDP to Bl:B2 mixtures gave a progressive decrease in Bl activity until 100% inactivation was obtained when approximately 0.2 mol of CeDP had been added per mol of Bl (see "Experimental Procedure"). UcDP gave results similar to CeDP. In the absence of Bl no inactivation was observed, and dithiothreitol could not substitute for Bl. The activity of protein Bl was not decreased by CeDP. In the absence of dithiothreitol, stoichiometric amounts of Bl were required for inactivation, while with dithiothreitol catalytic amounts of Bl sufficed. Only Bl with oxidation-
reduction active dithiols could fulfill this function, since B2 was not inactivated in the presence of B1 in which the dithiols had been oxidized by preincubation with CDP (2), with subsequent removal of the CDP by Sephadex G-50 chromatography. This result indicated that reduction of CzDP was required for inactivation of B2.

**CDP Protects Protein B2 against CzDP-inactivation**

Increasing amounts of CDP together with a constant amount of CzDP resulted in a decreased inactivation of B2, indicating competition between the analogue and the normal substrate for the same site on ribonucleotide reductase (see "Experimental Procedure"). It was difficult to measure the initial rates of CDP reduction because the curves representing NADPH oxidation leveled off within minutes due to complete inactivation of B2. CzMP gave no inactivation of B2 at concentrations at which complete inactivation of B2 by CzDP would have occurred. This is further support for the specificity of the CzDP reaction.

**Influence of Effectors on Inactivation by CzDP**

Ribonucleoside diphosphate reductase had to be in an active conformation in order to be inactivated by CzDP. Addition of the negative effector dATP slowed down the inactivation, while the further addition of ATP increased inactivation (data not shown).

**Reaction Products Formed from the CzDP**

When the B1-B2 complex was incubated with an excess of [8-32P]CzDP, analyses of the reaction mixture after complete inactivation of B2 showed the loss of 2.3 mol of sulphydryl from B1. After chromatography on Sephadex G-50 all radioactivity was found in the included volume, demonstrating the absence of protein-bound 32P. About 5% of the [8-32P]CzDP had been transformed to 2-deoxyribose 5'-[8-32P]diphosphate, as shown by thin layer chromatography. From the stoichiometry of this experiment one can calculate that about 2.8 mol of sulphydryl groups in B1 were oxidized/mol of deoxyribose diphosphate formed, and that about 1.5 mol of CzDP were converted/mol of B2.

Further analyses of the deproteinized incubation mixture by thin layer chromatography allowed the exclusion of 2'-deoxy-2'-aminocytidine 5'-diphosphate as a reaction product, while the presence of dCDP could not be excluded, since no system capable of separating CzDP from dCDP was available.

We conclude that addition of CzDP to a B1/B2 mixture resulted in oxidation of sulphydryl groups in B1, inactivation of B2, and probably the release of cytosine.

**Mechanism of CzDP-inactivation of B2**

The characteristic 410 nm absorbance peak of protein B2 due to the free radical (5) had been lost completely when the spectrum of a mixture of B1 and inactivated B2 was recorded after removal of low molecular weight components on a Sephadex G-25 column. Iron analyses showed that this was not due to loss of iron, since the inactivated B2 contained normal amounts of iron (5). Instead, it suggested that the free radical of B2 had been destroyed.

In order to test this hypothesis, protein B2 (specific activity 14,000 units/mg) was mixed with B1 and inactivated completely with CzDP (see "Experimental Procedure"). This led to the disappearance of the 410 nm absorbance peak. The radical in protein B2 can be regenerated by a process involving removal of the iron followed by its replacement (5). When the incubation mixture containing CzDP-inactivated B2 was treated in this way (see under "Experimental Procedure"), B2 regained its activity (specific activity 11,000 units/mg) and the 410 nm absorbance.

From this experiment it is concluded that inactivation of B2 by CzDP is due to the selective destruction of the free radical necessary for B2 activity.

**Effects of CzDP and CzTP on Other Ribonucleotide Reductases**

Ribonucleotide Reductase from Lactobacillus leichmannii and Calf Thymus Are Both Inactivated by Azido Derivative—Preincubation of the Lactobacillus leichmannii enzyme in 5 x 10^{-4} to 2.5 x 10^{-3} M CzTP in the presence of Mg^{2+}, dATP, dithiothreitol, and adenosine-5'-phosphate for 10 min at 37°C resulted in a more than 98% inactivation of the enzyme. This was not the result of competition between CTP and CzTP in the assay, since the same degree of inactivation was obtained when low molecular weight components were removed from the preincubation mixture by chromatography on Sephadex G-25 before the enzyme was assayed.

Preliminary experiments with a calf thymus ribonucleotide reductase preparation with a specific activity of 8 units/mg of protein showed that preincubation in 4 x 10^{-4} M CzTP for 5 min at 25°C resulted in a more than 98% inactivation.

**DISCUSSION**

Both CclDP and UcIDP are quite stable compounds. The only reaction observed in solution is their hydrolysis to the arabinonucleoside diphosphates but this reaction occurs slowly and only about 10% CclDP is transformed in 24 hours at pH 8 and 37°C (22). The analogs do not irreversibly inactivate other enzymes tested such as polynucleotide phosphorylase and DNA-dependent RNA polymerase from Escherichia coli. Neither CclDP nor CzTP react with protein B1 or B2 alone or with dithiothreitol. The azido derivatives can be reduced chemically to the corresponding 2'-NH₂ compounds; these do not inactivate ribonucleotide reductase but function as competitive inhibitors.¹

Both the 2'-chloro and the 2'-azido 2'-deoxyribonucleoside diphosphates are bound to the substrate binding site of the B1-B2 complex, as shown by the influence of allosteric effectors on the inactivation, protection of the enzyme by substrate, the inability of the monophosphates to act as inhibitors, and the requirement for the presence of both B1 and B2 to achieve inactivation.

In inactivating ribonucleotide reductase the chloro and azido derivatives behaved like a group of irreversible enzyme inhibitors called k_{cat} inhibitors. These are characterized as unreactive compounds (proinhibitors) which are converted to a highly reactive form (inhibitor) within an active site by the specific action of a particular enzyme (23). Once formed, the reactive form can interact directly with a crucial group in the active site of the enzyme and cause inactivation, or the enzyme-inhibitor complex can dissociate before such a reaction has occurred. The more effective the inhibitor, the more often it does react and inactivate the enzyme before dissociation occurs. This can be determined by measuring the percentage of enzyme inactivated when a stoichiometric amount of proinhibitor is added.

¹ Refer to the original text for further details on the specific compounds and their interactions.
CzDP was a very effective proinhibitor, since it inactivated protein B2 stoichiometrically. The value of about 0.2 mol of CzDP required for total inactivation of one mol of B2 agrees with the known variable and low content of free radical in the B2 preparations (24). CzDP was somewhat less effective, giving about 50% inactivation of B1 on addition of stoichiometric amounts of chloro derivative (2 mol of CzDP/mol of B1 based on two binding sites for ribonucleoside diphosphates/mol of B1).

The inactivation of B1 by the chloro derivatives required the presence of active B2 which could not be replaced by either apoB2 or hydroxylamine-inactivated B2, in spite of the fact that both of these modified forms can form a 1:1 complex with B1 as judged from sucrose gradient centrifugations. This indicates that a reaction product of the chloro derivative and not the compound itself inactivated B1.

The inactivation of B1 and disappearance of titrable sulfhydryl groups was correlated in time with the decomposition of the chloro derivatives. By varying the molar ratio of B1 to B2 in the incubation mixtures it was found that the number of sulfhydryl groups disappearing was always correlated to the amount of B1 (cf. Ref. 2). The sulfhydryl groups could not be regenerated by treatment with dithiothreitol, nor could they be recovered as cysteic acid after performic acid oxidation, excluding a straightforward oxidation of cysteine residues to cysteine as found on addition of normal ribonucleoside diphosphates to ribonucleotide reductase in the absence of reducing agents (2). Instead, the data strongly suggest a more extensive modification of the oxidation-reduction active dithiols. The nature of this modification is not known, but it is assumed to cause the irreversible inactivation of B1.

The increase in the absorption at 320 nm was not directly correlated with inactivation. The increase was a slow process and also continued after complete inactivation of B1. The inactivation of protein B2 by the azido derivative was due to a destruction of the free radical, since removal and readdition of iron—a procedure normally used to regenerate the free radical of B2 (5)—resulted in almost complete recovery of B2 activity.

Reactivation also resulted in the reappearance of the characteristic radical absorption at 410 nm which was lost during inactivation.

In agreement with the kcat concept, the following data suggest that a derivative formed from the azido nucleotide by reduction with ribonucleotide reductase rather than the nucleotide itself caused inactivation of B2. (a) Addition of CzDP to the B1-B2 complex caused a limited oxidation of sulfhydryl groups in B1 similar to addition of normal substrate with a simultaneous inactivation of B2, (b) B2 inactivation was not observed with B1 containing disulfides instead of active dithiols, and (c) S2 inactivation required the presence of stoichiometric amounts of B1 or B1 plus dithiothreitol.

It was shown earlier that the destruction of the free radical of B2 by hydroxylamine or hydroxyurea inactivated the enzyme, suggesting, but not proving, the requirement of the radical for the catalytic process (5). The destruction of the radical accompanying reduction of the azido derivatives by the B1-B2 complex is, on the other hand, a direct indication for the participation of the radical during ribonucleotide reduction.

The data presented in this paper show that the chloro and the azido derivatives inactivate one subunit each of ribonucleotide reductase, but only in the presence of the other subunit. This, plus the fact that ribonucleoside diphosphates bind exclusively to B1, shows that the active site of ribonucleotide reductase must be formed from both subunits.

The modification of the active dithiols of B1 by the chloro derivatives during B1 inactivation shows that the dithiols are located close to the ribonucleoside diphosphate binding site.

Other work (2) has shown that ribonucleotide reductase acts by a ping-pong mechanism, which means that the enzyme alternates between two stable forms during catalysis, the dithiol form of B1 and the disulfide form. It was also shown earlier (2) that electrons pass readily from the active dithiols in thioredoxin to those of B1 and that during normal ribonucleotide reduction the active dithiols of B1 are continuously regenerated by thioredoxin. This means that thioredoxin also interacts close to the ribonucleoside diphosphate binding site, but as a consequence of the ping-pong mechanism, thioredoxin leaves the enzyme before the ribonucleotide enters.

Earlier data (2) and those presented in this paper can be summarized in the model of the active site of ribonucleoside diphosphate reductase presented in Fig. 6. The site is formed both from B1 and B2, and it contains active dithiols contributed by B1 and a free radical contributed by B2. The active dithiols donate the electrons required for ribonucleotide reduction, while the function and nature of the free radical participating during catalysis is still unknown. The conformation of the active site is controlled by nucleoside triphosphate effectors which bind to B1 at sites distinct from the ribonucleoside diphosphate sites.

The azido derivative also inactivated the ribonucleoside triphosphate reductase from Lactobacillus leichmannii and a ribonucleotide reductase from calf thymus. In the case of the lactobacillus enzyme, evidence for the involvement of a free radical intermediate in the reductase reaction has been obtained by spectrophotometric stopped flow studies and EPR measurements (1). The inactivation by CzTP supports these observations, and the general sensitivity of ribonucleotide reductases for the azido derivative indicates similar reaction mechanisms with participation of free radicals.

Preliminary experiments have shown that 2'-deoxy-2'-azidocytidine can be used to reversibly inhibit DNA synthesis in vivo in tissue-cultured cells, and this inhibition is caused by a specific inactivation of ribonucleoside reductase.

REFERENCES

4 L. Skoog and L. Thelander, to be published.
Active site of ribonucleoside diphosphate reductase from Escherichia coli.
Inactivation of the enzyme by 2'-substituted ribonucleoside diphosphates.
L Thelander and B Larsson


Access the most updated version of this article at http://www.jbc.org/content/251/5/1398

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/251/5/1398.full.html#ref-list-1