threitol were not transferred via thioredoxin.

The inhibition studies with hydroxyurea and 2′-deoxy-2′-azidocytidine diphosphate suggested that the thymus enzyme might not permanently contain a free radical species but instead formed such a species only during the reaction. However, conclusive results regarding the reaction mechanism and catalytic site of mammalian reductases will require homogeneous preparations of the iron-binding subunit and an understanding of the conditions needed for recombination of the two proteins into an active enzyme complex.

References

Abrams, R., Libenson, L., & Edmonds, M. (1960) *Biochem. Biophys. Res. Commun. 3*, 272–274.
Atkin, C. L., Thelander, L., Reichard, P., & Lang, G. (1973) *J. Biol. Chem. 248*, 7464–7472.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Brown, N. C., & Reichard, P. (1969a) *J. Mol. Biol. 46*, 25–38.
Brown, N. C., & Reichard, P. (1969b) *J. Mol. Biol. 46*, 39–55.
Brown, N. C., Eliasson, R. Reichard, P., & Thelander, L. (1969) *Eur. J. Biochem. 9*, 512–518.
Castellot, J. J., Miller, M. R., & Pardee, A. B. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 351–355.
Cory, J. G., Fleischer, A. E., & Munro, J. B., III (1978) *J. Biol. Chem. 253*, 2898–2901.
Eriksson, S., Åkerman, M., & Thelander, L. (1977) *Biochem. Soc. Trans. 5*, 739–741.
Eriksson, S., Thelander, L., & Åkerman, M. (1979) *Biochemistry 18* (following paper in this issue).
Fisher, P. A., & Korn, D. (1977) *J. Biol. Chem. 252*, 6528–6535.
Hoffman, P. J., & Blakley, R. L. (1975) *Biochemistry 14*, 4804–4812.
Hopper, S. (1972) *J. Biol. Chem. 247*, 3336–3340.
Hopper, S. (1978) *Methods Enzymol. 51*, 237–246.
Jovin, T. M., Englund, P. T., & Kornberg, A. (1969) *J. Biol. Chem. 244*, 2996–3008.
Klippenstein, G. L., & Cory, J. G. (1978) *Biochem. Biophys. Res. Commun. 83*, 252–258.
Knorre, D. G., Kurbatov, V. A., & Samukov, V. V. (1976) *FEBS Lett. 70*, 105–108.
Larsson, A. (1969) *Eur. J. Biochem. 11*, 113–121.
Larsson, A., & Reichard, P. (1966) *J. Biol. Chem. 241*, 2533–2539.
Luthman, M., Eriksson, S., Holmgren, A., & Thelander, L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2158–2162.
Moore, E. C. (1977) *Adv. Enzyme Regul. 15*, 101–114.
Moore, E. C., & Hurlbert, R. B. (1966) *J. Biol. Chem. 241*, 4802–4809.
Neal, M. W., & Florini, J. R. (1973) *Anal. Biochem. 55*, 328–330.
O'Farell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.
Reichard, P. (1958) *Acta Chem. Scand. 12*, 2048.
Thelander, L. (1973) *J. Biol. Chem. 248*, 4591–4601.
Thelander, L., & Reichard, P. (1979) *Annu. Rev. Biochem. 48*, 133–158.
Thelander, L., Larsson, B., Hobbs, J., & Eckstein, F. (1976) *J. Biol. Chem. 251*, 1398–1405.
Wright, J. A., & Lewis, W. H. (1974) *J. Cell. Phys. 83*, 437–440.

Allosteric Regulation of Calf Thymus Ribonucleoside Diphosphate Reductase†

Staffan Eriksson,* Lars Thelander, and Margareta Åkerman

ABSTRACT: A highly purified ribonucleotide reductase from calf thymus catalyzed the reduction of all four ribonucleoside diphosphates at almost identical rates. Substrate specificity is regulated by allosteric effects. The activities toward CDP and GDP were purified in parallel and the two nucleotides competed for the same catalytic site. Taken together the data show that the same enzyme also in mammalian cells can reduce all four ribonucleotides. In the absence of positive effectors, the enzyme was inactive with any ribonucleoside diphosphate. Reduction of CDP and UDP was stimulated by ATP, reduction of GDP by dTTP, and reduction of ADP by dGTP. Reduction of the purine ribonucleotides was further stimulated by ATP combined with dTTP or dGTP. dATP served as a general inhibitor whose negative effects could be reversed by ATP. Inhibition was also caused by dTTP or dGTP which, in stimulating reduction of a single substrate, inhibited reduction of the other three substrates at the same time. The general pattern of regulation is similar to that observed for the *Escherichia coli* enzyme, but the effector requirements were more distinct with the mammalian enzyme. Our results fully explain the variations in pools of deoxyribonucleoside triphosphates observed earlier for cells in tissue cultre upon exposure to certain inhibitors of DNA synthesis. They may also explain the mechanism behind two immunodeficiency diseases associated with an inherited deficiency in the enzymes adenosine deaminase or purine nucleoside phosphorylase.

Two types of ribonucleotide reductases have been studied in detail: the cobamide-dependent ribonucleoside *tri*phosphate reductase from *Lactobacillus leichmannii* and the ribonucleoside *di*phosphate reductase from *Escherichia coli* which contains nonheme iron (Thelander & Reichard, 1979). In both cases the same enzyme reduces all four ribonucleotides and the substrate specificity and overall activity of the enzyme are regulated by nucleoside triphosphates which act as allosteric effectors (Thelander & Reichard, 1979).

Studies on chick embryo homogenates (Reichard et al., 1961) first demonstrated a regulatory mechanism for the synthesis of deoxyribonucleotides from ribonucleotides.

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Preliminary characterization of the ribonucleotide reductase in Novikoff tumor cell extracts (Moore & Hurlbert, 1966) demonstrated a complex regulation of the reduction of the four ribonucleotides. The complexity of the regulation suggested the existence of four different enzymes. Several reports have since appeared which claimed that the reduction of the four ribonucleotides is carried out by different enzymes in mammalian cells (Moore & Hurlbert, 1966; Peterson & Moore, 1976; Cory et al., 1976; Lewis et al., 1978). However, all these studies were done with crude enzyme preparations containing kinase and phosphatase activities and, therefore, the results must be interpreted with caution.

We have obtained a highly purified ribonucleotide reductase from calf thymus and have studied this enzyme with regard to substrate specificity and regulation by nucleoside triphosphate effectors and with regard to the capacity of the same enzyme to reduce all four different ribonucleotide substrates.

Experimental Procedures

Materials. Radioactively labeled nucleotides were obtained from Schwarz BioResearch. [^{3}H]UDP was prepared by deamination of [^{3}H]CDP (Häggmark, 1962). Unlabeled nucleotides were purchased from Calbiochem or Sigma.

Polyethylenimine plates were obtained from Macherey-Nagel & Co. and washed with sodium chloride and water before treatment with boric acid (Schrecker et al., 1968). Phosphodiesterase (*Crotalus atrox* type IV) was purchased from Sigma and bacterial alkaline phosphatase from Worthington.

Calf thymus ribonucleotide reductase (after chromatography on dATP–Sepharose) was prepared as described in the preceding paper (Engström et al., 1979).

Assays. CDP Assay. The standard incubation mixture (Engström et al., 1979) was used with the following modifications: 0.25 μmol of ATP instead of 0.15 μmol; 0.75 μmol of $MgCl_2$ instead of 0.32 μmol; and the KCl was replaced by 1.25 μmol of Tris-Cl buffer, pH 7.6. The reaction was performed at 37 °C for 10 min. The optimal Mg^{2+} concentration was directly correlated to the effector concentration (Engström et al., 1979). Therefore, in some experiments the $MgCl_2$ concentration was different from the one noted above.

The reaction was stopped with 0.5 mL of 1 M perchloric acid, and the rest of the assay was run as described earlier.

UDP, GDP, and ADP Assays. The standard incubation conditions were as described for the CDP assay but [^{3}H]UDP, [^{3}H]GDP, or [^{3}H]ADP (specific activity 30 000 to 60 000 cpm/nmol) was used as substrate.

The reactions were stopped by heating for 2 min at 100 °C. After centrifugation (in the case of crude enzyme preparations), 5 μL of a solution containing phosphodiesterase (15 mg/mL) and alkaline phosphatase (5 mg/mL) was added, and the samples were incubated for 1 h at 37 °C. All nucleotides were degraded to nucleosides by this treatment as checked in a separate experiment. The incubation mixtures were again heated as above, and carrier nucleoside (0.1 μmol) was added. After centrifugation, 4 μL of the supernatant solution was applied to polyethylenimine plates pretreated with borate (Schrecker et al., 1968). The plates were developed in 0.1 M boric acid for 2 h. Ribonucleoside and deoxyribonucleoside spots were identified under UV light (R_f values approximately 0.1 and 0.5, respectively) and cut out, the nucleosides were eluted with 1 mL of 2 M HCl for 1 h at 37 °C, and radioactivity was counted after addition of 10 mL of Instagel. The fraction of total radioactive nucleosides as deoxyribonucleoside was determined and used in the calculation of nmol of product formed/min.

K_m and V_{max} Determinations. The conditions of the assays were chosen so that maximal conversion of substrate to product was less than 40%. The background level of deoxyribonucleotides in our substrate ribonucleotides (approximately 1%) was monitored in each experiment, and the value was subtracted from all figures.

Protein Determination. In the ammonium sulfate fraction protein was determined by the method of Lowry as modified by Jovin (Jovin et al., 1969) and in the purified preparations by the absorbance at 280 nm in a 1-cm cuvette, with the assumption that 1 mg of protein/mL corresponds to an absorbance of 1.0.

Results

Levels of Kinase and Phosphatase Activities in the Highly Purified Calf Thymus Ribonucleotide Reductase. The enzyme preparation was tested for the presence of contaminating nucleotide kinase and phosphatase activities under the conditions of the standard assay. No detectable formation of CMP or cytidine was observed when 25 nmol of [^{3}H]CDP and 2.5 μg of enzyme were incubated for 30 min at 37 °C and aliquots of the reaction mixture were analyzed by paper chromatography. The stability of added deoxyribonucleotide effectors, i.e., [^{3}H]dGTP and [^{3}H]dTTP (2.5 and 12.5 nmol), was also tested under the same conditions and no breakdown of the effectors was found. Addition of ATP (5 μmol) to the incubation mixture containing 25 nmol of CDP gave a conversion of approximately 10% of the CDP substrate to CTP.

Thus, our enzyme preparation contained only trace amounts of nucleoside diphosphate kinase activity and, therefore, the stability of added nucleotides was sufficient to make studies of the substrate specificity and allosteric regulation conclusive.

Ribonucleoside Diphosphates Are the Preferred Substrates. The calf thymus enzyme showed only 10% activity with CTP as substrate compared with the activity with CDP by using the standard incubation conditions (substrate concentration 5×10^{-4} M).

Regulation of the Reduction of the Different Substrates. The reduction of ribonucleotides by the calf thymus enzyme required the presence of a positive allosteric effector. No deoxyribonucleotides could be detected in the absence of effector even with high ribonucleotide concentrations.

CDP Reduction. The reduction of CDP specifically required the presence of ATP. Optimal concentrations were $2–5 \times 10^{-3}$ M. The V_{max} value at 5×10^{-3} M ATP was three times higher than the value at 1×10^{-3} M ATP but the K_m value (3.0×10^{-5} M) was the same at both concentrations of the effector. Other nucleoside triphosphates tested, i.e., dTTP, dGTP, dATP, and dCTP, did not substitute for ATP.

The effects of combining ATP with different deoxyribonucleoside triphosphates are shown in Figure 1. dATP, dTTP, and dGTP all inhibited enzyme activity, while dCTP had no effect. dATP was the most effective inhibitor.

Inhibition by dTTP and dGTP was not evident at high substrate concentration but became pronounced at substrate concentrations close to the K_m value, demonstrating a decreased affinity of the enzyme for CDP as a result of the presence of the deoxyribonucleoside triphosphates.

Inhibiton by low concentrations of dATP could be overcome at least partially by increasing the ATP concentration (Table I). However, more than a 1000-fold excess of ATP over dATP was required.

UDP Reduction. The reduction of UDP required the addition of ATP at concentrations of $2–5 \times 10^{-3}$ M. dTTP, dGTP, and dCTP (5×10^{-6} to 1×10^{-3} M) had no effect as positive effectors.

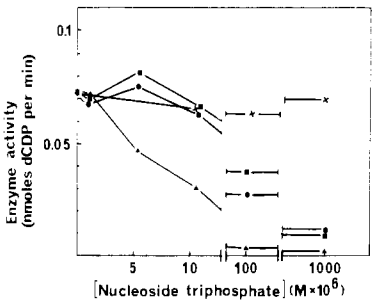

FIGURE 1: Effects of deoxyribonucleoside triphosphates on CDP reduction in the presence of ATP. Ribonucleotide reductase (9 μg), 5×10^{-5} M CDP, and 1×10^{-3} M ATP were used in each assay. The deoxyribonucleoside triphosphates added were dTTP (●—●), dGTP (■—■), dCTP (X—X), and dATP (▲—▲).

Table I: Competition between Effectors

substrate	effector 1	effector 2	act. (%)
CDP[a]		ATP (9×10^{-4} M)	100
	dATP (4×10^{-6} M)	ATP (9×10^{-4} M)	7
	dATP (4×10^{-6} M)	ATP (1.7×10^{-3} M)	10
	dATP (4×10^{-6} M)	ATP (5.2×10^{-3} M)	28
	dATP (4×10^{-6} M)	ATP (10.4×10^{-3} M)	45
GDP[b]	dTTP (2×10^{-5} M)		100
	dTTP (2×10^{-5} M)	dGTP (2×10^{-5} M)	75
	dTTP (2×10^{-5} M)	dGTP (1×10^{-4} M)	39
	dTTP (2×10^{-5} M)	dGTP (4×10^{-4} M)	23
	dTTP (2×10^{-5} M)	dGTP (4×10^{-4} M)	13
ADP[c]	dGTP (5×10^{-5} M)		100
	dGTP (5×10^{-5} M)	dTTP (5×10^{-5} M)	90
	dGTP (5×10^{-5} M)	dTTP (3×10^{-4} M)	48
	dGTP (5×10^{-5} M)	dTTP (6×10^{-4} M)	27
	dGTP (5×10^{-5} M)	dTTP (12×10^{-4} M)	13

[a] The CDP reduction was performed with 5.4 μg of enzyme and 5×10^{-5} M substrate. The ATP solution contained $MgCl_2$ (ATP: Mg^{2+} as 1:2), and no further $MgCl_2$ was added. One hundred percent activity corresponds to 0.07 nmol of product/min. [b] GDP (1×10^{-4} M), ATP (3×10^{-3} M), and enzyme (10 μg) were present in each assay. One hundred percent activity was 0.28 nmol of product/min. [c] The amount of enzyme was 5.1 μg and 5×10^{-5} M ADP and 4×10^{-3} M $MgCl_2$ were used. No ATP was added in this experiment. One hundred percent activity was 0.007 nmol of product/min.

The K_m value for UDP reduction at 5×10^{-3} M ATP was 1.0×10^{-4} M (data not shown).

In the presence of 1×10^{-3} M ATP, UDP reduction was inhibited by dATP, dGTP, and dTTP, with 50% inhibition at concentrations of 5×10^{-6} M dATP and 1×10^{-4} M dTTP or dGTP. Thus, the effects of nucleoside triphosphates on UDP reduction were very similar to those found for the CDP reduction.

GDP Reduction. Reduction of GDP required the presence of dTTP, while other nucleoside triphosphates, i.e., ATP, dATP, dGTP, or dCTP (5×10^{-6} to 1×10^{-3} M), had no effect as positive effectors (Figure 2). Combination of dTTP with ATP gave a twofold higher activity than dTTP alone. dGTP and ATP in combination did not stimulate GDP reduction (Figure 2).

Figure 3 shows a Lineweaver–Burk plot for GDP reduction with dTTP or a combination of dTTP and ATP as effectors. The K_m values determined from the figure are 5.0×10^{-5} M in both cases, demonstrating that the main effect of ATP was to increase the k_{cat} of the enzyme.

GDP reduction was inhibited by low concentrations of dATP (50% inhibition at 1×10^{-6} M) both with dTTP as positive effector and with a combination of dTTP and ATP. Also, dGTP inhibited the reaction. In the presence of fixed concentrations of dTTP and ATP, increasing concentrations of dGTP inhibitied GDP reduction (Table I).

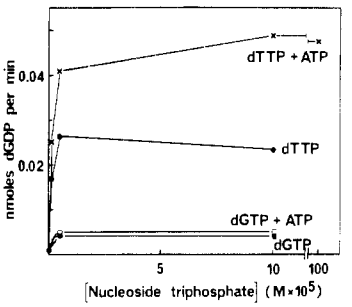

FIGURE 2: Effects of nucleoside triphosphates on GDP reduction. The assays were performed with 9 μg of enzyme and 5×10^{-5} M GDP. The effectors used were ATP (△), dTTP (●—●), dGTP (■—■), varying concentrations of dTTP in combination with 9×10^{-4} M ATP (X—X) or varying concentrations of dGTP in combination with 9×10^{-4} M ATP (□—□).

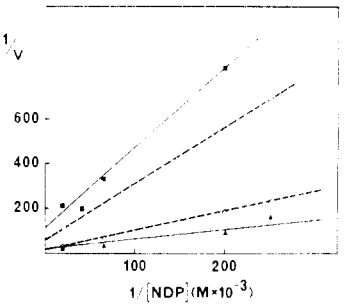

FIGURE 3: Lineweaver–Burk plot of GDP and ADP reduction. For GDP reduction (open symbols and broken lines), the reactions were performed with 4.5 μg of ribonucleotide reductase, and the effectors used were 5×10^{-5} M dTTP (○---○) or a combination of 5×10^{-5} M dTTP and 1×10^{-3} M ATP (△---△). For ADP reduction (filled symbols), the reactions were performed with 5.1 μg of enzyme, and the effectors used were 5×10^{-5} M dGTP (■—■) or a combination of 1.7×10^{-3} M ATP and 5×10^{-5} M dGTP (▲—▲). V = nanomoles of product produced per minute at 37 ° C.

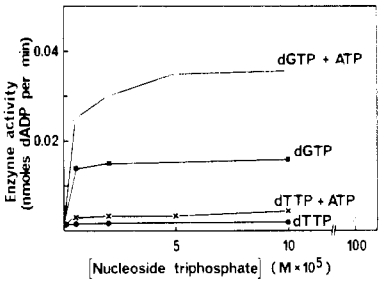

FIGURE 4: Effects of nucleoside triphosphates on ADP reduction. The amount of enzyme used was 9 μg and the effectors were ATP (△), dTTP (●—●), dGTP (■—■), varying concentrations of dTTP in combination with 1×10^{-3} M ATP (X—X), or varying concentrations of dGTP in combination with 1×10^{-3} M ATP (□—□).

ADP Reduction. The reduction of ADP specifically required dGTP. ATP, dATP, dTTP, and dCTP (5×10^{-6} to 1×10^{-3} M) showed no effect as positive effectors. A combination of ATP and dGTP gave a twofold higher activity than dGTP alone. ATP and dTTP did not stimulate (Figure 4).

A Lineweaver–Burk plot for ADP reduction is shown in Figure 3. The effectors used were dGTP or a combination of dGTP and ATP. The K_m value for the ADP reduction was 4.0×10^{-5} M in both cases.

ADP reduction was inhibited by dATP (with 50% inhibition at 5×10^{-6} M) by using dGTP (5×10^{-5} M) + ATP (1×10^{-3} M) as effectors. ADP reduction was also inhibited by

Table II: Summary of K_m and V_{max} Values for the Different Substrates of Purified Calf Thymus Ribonucleotide Reductase

substrates	K_m ($\times 10^{-5}$ M)	V_{max} (nmol min^{-1} mg^{-1})	effector
CDP	3.0–3.2	22.0	ATP (5×10^{-3} M)
UDP	10	14.2	ATP (5×10^{-3} M)
ADP	2.8–5.0	19.6	dGTP (5×10^{-4} M) + ATP (2×10^{-3} M)
GDP	4.3–5.0	18.5	dTTP (5×10^{-4} M) + ATP (2×10^{-3} M)

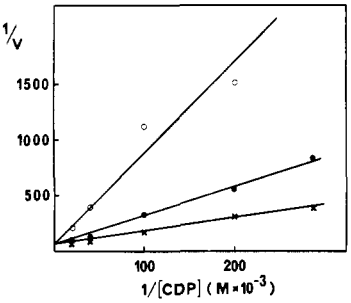

FIGURE 5: Competition between CDP and GDP as substrates for the enzyme. The amount of enzyme was 4.5 μg and the effectors used were dTTP (2×10^{-5} M) in combination with ATP (1×10^{-3} M). The [^{3}H]CDP concentration was varied in the presence of 3.9×10^{-6} M GDP (●—●), 19.7×10^{-6} M GDP (○—○), or without GDP addition (X—X).

increasing concentrations of dTTP, with 50% inhibition at a tenfold excess of dTTP over dGTP (Table I), indicating a competition between these effectors for the same site on the enzyme.

One Enzyme Reduces All Four Ribonucleotides. CDP and GDP Reductase Activities at Different Levels of Purification of the Calf Thymus Ribonucleotide Reductase. Ribonucleotide reduction was assayed either with CDP or GDP in the fractions from the standard purification (Engström et al., 1979). Incubation conditions were modified so that a larger volume (0.15 mL) could be used, and optimal concentrations of positive effectors were added, i.e., 3×10^{-3} M ATP for CDP reduction and 3×10^{-3} M ATP combined with 5×10^{-4} M dTTP for GDP reduction. The activities in the ammonium sulfate fraction, the DEAE-cellulose pool, and the hydroxylapatite pool were 0.01, 0.06, and 0.33 nmol of dCDP min^{-1} (mg of protein)$^{-1}$ and 0.01, 0.06, and 0.23 nmol of dGDP min^{-1} (mg of protein)$^{-1}$, respectively.

Thus, the ratio between the activities with the two substrates was almost constant in the different fractions, and no indication for a separation of different ribonucleotide reductases during the purification was found.

V_{max} and K_m for the Different Ribonucleotide Substrates of the Purified Enzyme. Table II shows the V_{max} and K_m for the different substrates of the enzyme in the presence of optimal concentrations of positive effector(s). Comparable maximal activities were found with all four substrates tested, although a slightly lower activity was found with UDP. The K_m values were also similar, except that UDP had a K_m value three times larger.

Competition between GDP and CDP for the Same Catalytic Site. The ability of GDP to compete with CDP as substrate for the enzyme was tested in the presence of dTTP (2×10^{-5} M) and ATP (1×10^{-3} M). This combination of effectors allowed both CDP and GDP reduction.

Figure 5 shows a Lineweaver–Burk plot for CDP reduction in the absence and in the presence of two different concentrations of GDP. There was a clear inhibition of CDP reduction by GDP, and this could be overcome by increasing the CDP concentration, demonstrating competition between the two substrates for the same site on the enzyme. The K_i value for GDP was 6.3×10^{-6} M as determined from Figure 5, and the K_m for CDP was 3.0×10^{-5} M in the same experiment.

Table III: Summary of the Stimulatory and Inhibitory Effects of Different Nucleoside Triphosphates on the Activity of Calf Thymus Ribonucleotide Reductase

substrates	positive effector[a] or effector combination	inhibition[b]
CDP	ATP (1×10^{-3} M)	dATP (5×10^{-6} M)
UDP		dTTP (1×10^{-4} M)
		dGTP (1×10^{-4} M)
GDP	dTTP (5×10^{-6} M) + ATP (1×10^{-3} M)	dATP (5×10^{-6} M) dGTP (5×10^{-5} M)
ADP	dGTP (5×10^{-6} M) + ATP (1×10^{-3} M)	dATP (5×10^{-6} M) dTTP (5×10^{-5} M)

[a] The concentrations given represent the values for half-maximal stimulation. [b] The concentrations of effectors which give half-maximal inhibition of the stimulated reaction. See also Table I.

Discussion

The reductase used for our studies was a preparation purified more than 3000-fold from calf thymus. Enzyme activity was dependent on two protein components. One, with the capacity to bind to dATP–Sepharose, constitutes the bulk of the total protein and the other, which contains iron, is present in low, nonstoichiometric amounts (Engström et al., 1979). Even though not homogeneous, the enzyme preparation contained no phosphatase and only trace amounts of a nucleoside diphosphate kinase. Therefore, for the first time, a detailed study could be made of the allosteric regulation of a mammalian ribonucleotide reductase.

The data firmly established that a single enzyme catalyzed the reduction of all four ribonucleoside diphosphates and that its substrate specificity and activity were controlled by allosteric effectors. These results resemble the situation in bacteria. Our conclusion is based on measurements of GDP and CDP reduction at different stages during enzyme purification, showing complete copurification of the two activities. Furthermore, all four ribonucleoside diphosphate substrates were reduced at comparable rates by the purified enzyme, and there was a direct competition between CDP and GDP for the same catalytic site. Also, the highly purified rabbit bone marrow reductase was reported to show comparable specific activities for the reduction of CDP, UDP, ADP, and GDP (Hopper, 1978).

The regulation of the substrate specificity and overall activity of the calf thymus reductase showed a very distinctive pattern (Table III). The presence of a positive effector was required for reduction of any ribonucleotide substrate. Reduction of CDP and UDP was only stimulated by ATP, reduction of GDP by dTTP, and reduction of ADP by dGTP. Purine ribonucleotide reduction was further stimulated by ATP but only in combination with dTTP and dGTP, respectively. dATP was a general inhibitor and the inhibition could be reversed by ATP.

In general, the allosteric regulation of the thymus reductase was similar to the regulation of the *E. coli* enzyme, but the effects were more distinct. By analogy with the bacterial enzyme (Brown & Reichard, 1969), the allosteric pattern of the thymus reductase might be explained by postulating two

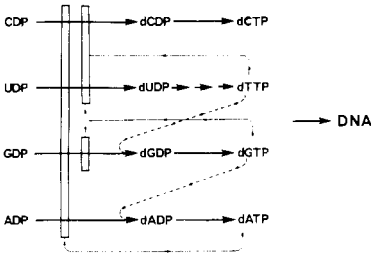

FIGURE 6: Scheme of the physiological regulation of deoxyribonucleotide synthesis. The broken arrows stand for positive effects, the open bars for negative effects. Note that for simplicity ATP is not included in the scheme, although the active form of the enzyme always binds ATP.

classes of effector binding sites on the enzyme. One class, capable of binding ATP, dTTP, or dGTP, then would regulate the substrate specificity (specificity site), while the other class, binding ATP or dATP, would regulate the overall activity (activity site). Preliminary binding experiments support this hypothesis (Engström et al., 1979). This arrangement would explain the stimulation by ATP of the purine ribonucleotide reduction already activated by dTTP or dGTP and the inhibition of ribonucleotide reduction caused by high concentrations of dTTP or dGTP. In the latter case, binding of dTTP or dGTP to the specificity site would induce a conformation of the catalytic site fitting only one specific substrate, thereby inhibiting the reduction of the other three substrates.

No stimulation or inhibition of the thymus reductase was observed by dCTP. This nucleotide therefore appears to lack the properties of an allosteric effector.

Our in vitro data can be integrated into a scheme that links ribonucleotide reduction to DNA synthesis (Figure 6). In this scheme, the active form of the enzyme always binds ATP. The synthesis of deoxyribonucleotides can then be represented as a sequential reaction starting with the reduction of CDP and UDP by the ATP-activated enzyme. The synthesis proceeds to GDP reduction via a dTTP + ATP activated enzyme, and, finally, reaches ADP reduction by a dGTP + ATP activated enzyme. Accumulation of dATP (e.g., in the absence of DNA synthesis) completely turns off the activity of the reductase. Accumulation of dTTP shuts off the reduction of pyrimidine substrates, while accumulation of dGTP in addition turns off GDP reduction. The inhibition by dATP is reversed by ATP and, therefore, the total activity of ribonucleotide reductase in the cell is controlled by the ATP/dATP ratio.

The validity for the in vivo situation of the scheme shown in Figure 6 can be tested by measuring deoxyribonucleoside triphosphate pools in tissue culture cells (Skoog et al., 1974). The inhibitory effects on DNA synthesis of hydroxyurea (Skoog & Nordenskjöld, 1971), thymidine (Bjursell & Reichard, 1973), or deoxyadenosine (Meuth & Green, 1974; Meuth et al., 1976) were all accompanied by changes in the intracellular pools of the deoxyribonucleotides. These changes can now be explained fully by the allosteric properties observed for the purified thymus reductase. Also a deoxyadenosine-resistant cell line was isolated and these cells contained a higher level of ribonucleotide reductase activity than normal cells, and the activity was less sensitive to inhibition by dATP (Meuth & Green, 1974).

Two hereditary immunodeficiency diseases in man may also ultimately be caused by an imbalance of the allosteric control of the reductase (Cohen et al., 1979). These patients show a deficiency in one or two purine salvage enzymes, adenosine deaminase or purine nucleoside phosphorylase, leading to abnormally high levels of dATP or dGTP in their lymphocytes. In model systems (Cohen et al., 1979; Ullman et al., 1979), this leads to a drastically decreased intracellular concentration of dCTP and to some extent dTTP, a result in full accordance with the allosteric regulation of the thymus ribonucleotide reductase (Figure 6). The low level of deoxypyrimidine triphosphates caused an inhibition of DNA synthesis and cell proliferation.

References

Bjursell, G., & Reichard, P. (1973) *J. Biol. Chem. 248*, 3904–3909.

Brown, N. C., & Reichard, P. (1969) *J. Mol. Biol. 46*, 39–55.

Cohen, A., Ullman, B., Gudas, L. J., & Martin, D. W., Jr. (1979) *Enzyme Defects Immune Dysfunction, Ciba Found. Symp. 68* (in press).

Cory, J. G., Mansell, M. M., & Whitford, T. W., Jr. (1976) *Adv. Enzyme Regul. 14*, 45–62.

Engström, Y., Eriksson, S., Thelander, L., & Åkerman, M. (1979) *Biochemistry 18* (preceding paper in this issue).

Häggmark, A. (1962) *Cancer Res. 22*, 568–572.

Hopper, S. (1978) *Methods Enzymol. 51*, 237–246.

Jovin, T. M., Englund, P. T., & Kornberg, A. (1969) *J. Biol. Chem. 244*, 2996–3008.

Lewis, W. H., Kuzik, B. A., & Wright, J. A. (1978) *J. Cell Physiol. 94*, 287–298.

Meuth, M., & Green, H. (1974) *Cell 3*, 367–374.

Meuth, M., Aufreiter, E., & Reichard, P. (1976) *Eur. J. Biochem. 71*, 39–43.

Moore, E. C., & Hurlbert, R. B. (1966) *J. Biol. Chem. 241*, 4802–4809.

Peterson, D. M., & Moore, E. C. (1976) *Biochim. Biophys. Acta 432*, 80–91.

Reichard, P., Canellakis, Z. N., & Canellakis, E. S. (1961) *J. Biol. Chem.* 236, 2514–2519.

Schrecker, A. W., Jacobsen, D. W., & Kirchner, J. (1968) *Anal. Biochem.* 26, 474–477.

Skoog, L., & Nordenskjöld, B. (1971) *Eur. J. Biochem. 19*, 81–89.

Skoog, L., Bjursell, G., & Nordenskjöld B. (1974) *Adv. Enzyme Regul. 12*, 345–354.

Thelander, L., & Reichard, P. (1979) *Annu. Rev. Biochem. 48*, 133–158.

Ullman, B., Gudas, L. J., Clift, S. M., & Martin, D. W., Jr. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1079–1083.