Ribbonucleotide Reductase from Euglena gracilis, a Deoxyadenosylcobalamin-dependent Enzyme*

(Received for publication, April 13, 1970)

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SUMMARY

A ribonucleotide reductase requiring 5'-deoxyadenosylcobalamin has been detected in Euglena gracilis var. bacillaris by measuring T2H exchange from labeled coenzyme to water. Extracts were partially purified by centrifugation, dialysis, and treatment with MnCl2. These extracts catalyzed the reduction of ribonucleoside triphosphates to the corresponding deoxyribonucleotides as measured by colorimetric assay with the diphenylamine procedure. The enzyme required 5'-deoxyadenosylcobalamin, a dithiol, and a ribonucleoside triphosphate for exchange activity and reduction. Monothiols could not replace the dithiol requirement. Ribonucleoside diphosphates were slightly active as substrates in both assay systems. Mg++, at low concentrations, did not affect the reaction; at concentrations greater than 2 mM, this ion was inhibitory. The reduction of ribonucleotides was inhibited by adding deoxyribonucleotides other than the immediate products; e.g., ATP reduction was inhibited by adding dGTP, dCTP, or dUTP, but not by dATP. dTTP inhibited the reduction of all ribonucleotides to the greatest extent. The molecular weight of this enzyme, determined by sucrose density gradient centrifugation, was approximately 145,000.

Extracts of Ochromonas malhamensis and Ochromonas danica showed no activity in the 3H exchange assay under similar conditions.

Blakley (1) and co-workers have isolated a ribonucleotide reductase from Lactobacillus leichmannii, which requires 5'-deoxyadenosylcobalamin for activity and which reduces only the ribonucleoside triphosphates at an appreciable rate. Cell-free extracts of Rhizobium meliloti also contain a deoxyadenosylcobalamin-dependent ribonucleotide reductase (2); ribonucleoside diphosphates are the preferred substrates for this enzyme. In a preliminary study, Blakley et al. (3) have also shown that a number of strains of Lactobacillus acidophilus have a high level of deoxyadenosylcobalamin-dependent reductase activity, while Abeles and Beck (4) were able to show that cell-free extracts of Clostridium sticklandii and Clostridium tetanomorphum catalyze tritium exchange between 5'-deoxyadenosylcobalamin-5-3H2 and water. This hydrogen exchange reaction is considered to be an integral part of the reduction and thus serves as a qualitative assay for a deoxyadenosylcobalamin-requiring ribonucleotide reductase. The evidence accumulated thus far indicates that the cobalamin-dependent reductase occurs in several different types of microorganisms. On the other hand, ribonucleotide reduction in mammalian systems and in several microbial preparations is not stimulated by 5'-deoxyadenosylcobalamin (5). The requirements for the mammalian systems appear to be very similar to those established for the Escherichia coli reductase by Reichard (6) and co-workers.

Both Euglena gracilis and Ochromonas malhamensis require vitamin B12 for growth. These algae are routinely used in microbiological assays for vitamin B12 and other cobalamins in blood and various materials. Whereas the requirement of a cobalamin in L. leichmannii is spared by deoxyribonucleosides, E. gracilis grown in the absence of a cobalamin does not respond to either deoxyribosides or methionine (6). However, vitamin B12 depletion in both L. leichmannii and E. gracilis leads to the formation of abnormally large cells and prolongation of the generation time (7, 8), suggesting that in both organisms vitamin B12 participates in DNA synthesis. In contrast, the cobalamin requirement of O. malhamensis is spared by methionine and not affected by deoxyribonucleosides.

By using the extremely sensitive hydrogen exchange reaction between 5'-deoxyadenosylcobalamin-5-3H2 and water, the presence of a cobalamin-requiring ribonucleotide reductase has been detected in extracts of E. gracilis. This communication describes several properties of this partially purified ribonucleotide reductase from E. gracilis.

EXPERIMENTAL PROCEDURES

Materials—Nucleotides were purchased from P-L Biochemicals. DL-Lipoic acid, dithiothreitol, dithiocysteine, pig muscle lactate dehydrogenase, and pig heart diaphorase were obtained from Sigma. Pig heart malate dehydrogenase was purchased from Boehringer. Dihydrolipoate was prepared by reduction of lipoic acid (9) without distillation of the product. 5'-Deoxyadenosylcobalamin-5-3H2 (20 μCi per μmole) was synthesized by the method of Gleason and Hogenkamp (10). Ribonucleotide reductase from L. leichmannii was kindly supplied by Dr. R. L. Blakley; this preparation has a specific activity of 70 μmoles per hour per mg of protein. Cultures of E. gracilis var.
bacillaris, O. malhamensis, and Ochromonas danica were a gift of Dr. Clive Bradbeer.

Methods—E. gracilis was maintained on a semisolid medium (11) at room temperature and subcultured every month. To obtain large quantities of cells, the algae were grown in 2800-ml Fernbach flasks containing 1 liter of the following medium: 1 g of bovine extract (Baltimore Biological Laboratory), 2 g of Difco tryptone, 2 g of Difco yeast extract, and 0.01 g of calcium chloride. The medium was adjusted to approximately pH 6 with 5 N HCl. The flasks were autoclaved twice and inoculated either from 15-ml culture tubes or with approximately 100 ml of actively growing cells. The cultures were grown in continuous illumination with banks of fluorescent lamps at 18-20°. Magnetic stirrers were used to agitate the medium. O. malhamensis and O. danica were grown in a similar manner with the following medium: 1.0 g of glucose, 1.0 g of Bacto-tryptone, 1.0 g of Difco yeast extract, 40 ml of Difco liver infusion, and 960 ml of distilled water. E. gracilis was also grown holophytically in a defined medium (12). The cultures were continuously flushed with a mixture of 5% CO₂ and air under sterile conditions. In all cases, cell growth was followed by recording the absorbance at 650 nm. The cells were harvested in the logarithmic phase (absorbance at 650 nm approximately 1.5).

Preparation of Cell-free Extract—All operations were carried out at 4°. The cells were harvested by centrifugation at 8000 × g and suspended in a solution containing 0.01 M Tris-HCl buffer (pH 7.4), 0.005 M mercaptoethanol, and 0.3 M sucrose. The cells were again sedimented at 8000 × g and resuspended in 70 ml of the above solution but without the sucrose. Six liters of culture yielded about 10 g of packed cells. A small amount of neutral Norit-A charcoal (approximately 50 mg per ml of cell suspension) was added. The cells were then disrupted by sonic oscillation for 10 min in a Raytheon 10 KC sonic oscillator. The cell debris and charcoal were removed by centrifugation at 37,000 × g for 15 min. These crude extracts usually contain 20 to 25 mg of protein per ml and can be used in the tritium exchange assay without further treatment. This crude extract slowly loses activity when stored at -10°.

For further purification, the cell-free extract was centrifuged at 29,000 × g for 1.5 hours. The supernatant was removed and dialyzed for 48 hours against several changes of 0.01 M Tris-HCl buffer, pH 7.4, containing 0.005 M mercaptoethanol. The dialyzed extract was then treated with MnCl₂ to remove chlorophyll and nucleoproteins. The nucleic acid concentration was estimated from the absorbance at 260 nm (E₂₆₀(λ) = 20) and 0.005 ml of a 1 N MnCl₂ solution was added per mg of nucleic acid. 12000 × g for 10 min. The resulting supernatant was dialyzed for 24 hours against 0.05 M sodium dimethylglutarate buffer, pH 7.2, containing 0.005 M mercaptoethanol. This partially purified extract can be stored at 4° and remains active for approximately 1 month.

Assay Procedure—Two procedures were used to assay ribonucleotide reductase activity. In Method 1, ribonucleotide reductase activity was determined by measuring tritium exchange between 5'-deoxyadenosylcobalamin-5'-H₂ and water. This procedure provides a very specific and sensitive measure of 5'-deoxyadenosylcobalamin-requiring ribonucleotide reductase (4, 13). Reaction mixtures contained 0.12 M sodium 3,3-dimethylglutarate buffer (pH 7.2), 1.2 mg EDTA, 26 mM dihydropropionate or other dithiol, 2 mM nucleotide, 0.01 mM 5'-deoxyadenosylcobalamin-5'-H₂, and 1 to 4 mg of E. gracilis preparation (0.050 to 0.1 ml) in a total volume of 0.5 ml. The enzyme was added to the assay mixture in dim light, and the reaction was initiated by the addition of enzyme. The assay tubes were incubated in the dark at 37°, and the reaction was terminated by freezing in liquid nitrogen. Water was removed from the tubes by sublimation, and a 0.1-ml aliquot of the thawed sublimate was added to 10 ml of scintillation fluid (14). Radioactivity was determined in a Packard model 3003 Tri-Carb liquid scintillation spectrometer. Specific activity is defined as the radioactivity released to the solvent (counts per min) per mg of protein after 30-min incubation.

In Method 2, ribonucleotide reductase activity was determined by measuring the amount of deoxyribonucleotide formed by the diphenylamine procedure of Blakley (15). The composition of the reaction mixture was the same as that described in Method 1, except that unlabeled 5'-deoxyadenosylcobalamin was used. When assaying for the reduction of purine nucleotides, the reaction was stopped by placing the tubes in ice and adding 0.4 ml of 0.5 M chloroacetamide in 0.25 mM potassium phosphate buffer, pH 7.3. The tubes were heated for 10 min in a boiling water bath. After cooling the tubes, 2 ml of diphenylamine reagent were added. The mixtures were then incubated at 37° for 4 hours, and the absorbance at 585 nm was determined. The amount of product formed was estimated from standard curves for dATP or dGTP. In assaying for the reduction of pyrimidine nucleotides, the reaction was terminated by adding 0.2 ml of 1 N HCl. The precipitated protein was removed by centrifugation at 50 × g for approximately 5 min. The supernatant solution was extracted twice with 2 ml of petroleum ether to remove lipase acid, and the ether phase was discarded. The solution was then mixed with 0.2 ml of saturated bromine water and allowed to react for 10 min; 0.050 ml of 5 N NaOH was added and the solutions were allowed to stand for 10 min for dCMP estimation or 40 min for dUMP estimation. Two milliliters of diphenylamine reagent were then added, and the tubes were incubated for 4 hours at 50° for color production with dCMP or at 37° with dUMP. Absorbance at 585 nm was determined and the amount of product was estimated from standard curves for the respective nucleotides.

Protein concentration was determined by the biuret method (16).

RESULTS

Growth Conditions—In general, cells harvested in the mid to late logarithmic growth phase showed the highest enzyme activity, whereas extracts of cells harvested in the early logarithmic growth phase were only slightly active. Extracts prepared from cells harvested in the stationary phase were inactive. The effect of specific growth conditions on the level of ribonucleotide reductase activity as measured by Method 1 is shown in Table 1. Crude extracts of holophytically grown cells of E. gracilis had no activity as measured by this assay procedure. However, after exhaustive dialysis against 0.01 M Tris-HCl buffer, pH 7.4, these extracts were able to catalyze the exchange reaction. Extracts of cells grown heterotrophically in the dark have approximately the same level of enzyme activity as light-grown cells. In all cases, the activities are only about 2% of the level of ribonucleotide reductase in lysates of L. leichmannii.

Crude extracts of either O. malhamenis, a chrysomonad which like E. gracilis requires vitamin B₁₂ for growth, nor O.


danica, a chrysomonad which does not require a cobalamin, catalyzed the hydrogen exchange reaction. Even after extensive dialysis, these preparations were not active.

Because the ribonucleotide reductase activity of crude extracts of E. gracilis was very low, the enzyme could be detected only by the extremely sensitive exchange reaction. Initial attempts to measure deoxyribonucleotide formation by the diphenylamine procedure (Method 2) or the isotope method of Bertani, Haggmark, and Reichard (17) were unsuccessful. The more active preparations used in the following procedures were obtained by inoculating the Fernbach flasks with 100 ml of a culture containing cells in the mid logarithmic growth phase.

**Effect of Growth Conditions on Ribonucleotide Reductase Activity**

Crude extracts were prepared as described in the text and assayed by assay Method 1. Reaction mixtures contained 0.12 M dimethylglutarate buffer (pH 7.2), 1.2 mM EDTA, 26 mM dihydro-lipoate, 2 mM ATP, 0.01 mM 5'-deoxyadenosylcobalamin-5'-H₂ (specific activity 20 μCi per μmol), and 0.2 ml of cell-free extract in a total volume of 0.5 ml. The reaction mixtures were incubated at 37° for 30 min.

| Organism and growth condition | Tritium released to the solvent (cpm/mg protein) |
|-------------------------------|-----------------------------------------------|
| **Euglena gracilis**          |                                               |
| Heterotrophic in the light    | 340                                           |
| Holophytic                    | 0                                             |
| Holophytic (cell extract dialyzed) | 380                                         |
| Heterotrophic in the dark     | 260                                           |
| **Ochromonas malhamensis**    |                                               |
|                               | 0                                             |
| **Ochromonas danica**         |                                               |
|                               | 0                                             |
| **Tetradanilla leichmannii**  |                                               |
|                               | 21,000                                        |

**Purification of ribonucleotide reductase**

Assay conditions are described in the text and in Table I; 26 mM dithiothreitol was used as reducing agent.

| Fraction             | Specific activity | Total activity |
|----------------------|-------------------|----------------|
| Crude extract        | 850               | X 10⁴          |
| Centrifugation and dialysis | 7,200       | 6.4            |
| MnCl₂ treatment and dialysis | 14,200      | 6.5            |

**Requirements for ribonucleotide reductase activity**

Assay conditions are described in the text and in Table I; 26 mM dithiothreitol was used as reducing agent. The partially purified reductase was prepared from Euglena gracilis started from a 10% inoculum and grown heterotrophically in the light.

| Reaction system       | Tritium exchange (cpm/mg protein) | Reduction (μmoles dATP/mg protein) |
|-----------------------|-----------------------------------|-----------------------------------|
| Complete system       | 38,500                            | 68                                |
| Omissions             |                                    |                                   |
| ATP                   | 746                                | 0                                 |
| Dithiothreitol        | 2,440                              | 0                                 |
| 5'-Deoxyadenosineylcobalamin | 0                              | 0                                 |
| Enzyme                | 135                                | 0                                 |

**FIG. 1.** Effect of Mg²⁺ concentration on the rate of ATP reduction by ribonucleotide reductase of Euglena gracilis. Assay conditions are described in Table III, except that EDTA was omitted from the reaction mixtures.
**TABLE V**

| Nucleotide used | Tritum exchange | Reduction |
|-----------------|-----------------|-----------|
|                 | cpm/mg protein  | pmole deoxyribonucleotide/mg protein |
| ATP             | 38,500          | 68        |
| ADP             | 5,500           | 5         |
| AMP             | 500             | 0         |
| dATP            | 13,900          | 86        |
| GTP             | 36,200          | 48        |
| GDP             | 29,000          | 9         |
| GMP             | 940             | 0         |
| dGTP            | 36,000          | 35        |
| ITP             | 26,600          | 35        |
| UTP             | 17,900          | 35        |
| UDP             | 700             | 0         |
| UMP             | 455             |           |
| dUTP            | 3,540           |           |
| CTP             | 16,700          | 39        |
| CDP             | 1,200           | 9         |
| dCTP            | 5,000           |           |
| dTTP            | 680             |           |

**Effect of Deoxyribonucleotides**—The ribonucleotide reductase of *E. gracilis* catalyzes the reduction of the five ribonucleoside triphosphates at markedly different rates (18). However, in the presence of specific deoxyribonucleotides, the relatively low rates of ATP and CTP reduction are increased to the rate of GTP reduction. A similar study for the ribonucleotide reductase of *E. gracilis* shows several fundamental differences between these two reductases (see Table VI).

Unlike the bacterial enzyme, the reductase of *E. gracilis* reduces all four substrates at approximately the same rate. In addition, the rate of reduction is not stimulated by a specific deoxyribonucleotide, but rather the reduction of a particular purine ribonucleotide is inhibited by all deoxyribonucleotides except its own product. Thus, as shown in Table VI, the reduction of GTP is inhibited by dATP, dCTP, dUTP, and dTTP but is not affected by dGTP. On the other hand, the reduction of ATP is not affected by dATP but is inhibited by dGTP, dCTP, dUTP, and dTTP. dTTP was found to be the most effective inhibitor of both reduction and exchange. It was the only deoxyribonucleotide that inhibited the reduction of all ribonucleotides.

Recently, Brown et al. (19) have shown that the ribonucleotide reductase from *E. coli* is either inhibited or stimulated by dATP, depending on the concentration of the deoxyribonucleotide used. To test for such a dual effect in the *E. gracilis* ribonucleotide reductase, the inhibition by two deoxyribonucleotides, dITP and dGTP, was studied in greater detail. These two deoxyribonucleotides were found only to inhibit the reduction of ATP; the extent of inhibition was directly proportional to the amount of deoxyribonucleotide added, over a concentration range of 10^{-6} to 10^{-2} M. Technical limitations of the colorimetric assay precluded the determination of meaningful kinetic constants for this inhibitory effect.

**Estimation of Molecular Weight**—The molecular weight of the partially purified ribonucleotide reductase from *E. gracilis* was estimated by sucrose density gradient centrifugation. Linear sucrose gradients, 5 to 20%, with a total volume of 4.6 ml were prepared by the technique described by Martin and Ames (20) except that the mixing chamber was agitated by a magnetic bar rather than a stirring rod. Sucrose was buffered with 0.05 M dimethylglycine, pH 7.2, containing 0.005 M mercaptoethanol. Approximately 5 to 7 mg of a partially purified extract from *E. gracilis* as well as varying amounts of standards (total protein less than 10 mg) were layered on the gradients. The gradients were centrifuged in a SW 50.1 rotor for 12 hours in a Spinco model L ultracentrifuge at 140,000 × g. The rotor was decelerated without brake. The bottom of the gradient tubes was punctured and drops were collected to give approximately 25 fractions per gradient. The fractions were assayed for enzyme activity. Commercial preparations of rabbit muscle lactic dehydrogenase, pig heart malate dehydrogenase, and pig heart phosphoglycerase were used as standards. These enzymes were assayed by published techniques (References 21, 22, and 23, respectively). *L. leichmannii* ribonucleotide reductase, also a standard, and the *E. gracilis* enzyme were assayed by the 3H tritium exchange procedure. A typical sedimentation pattern can be seen in Fig. 2. The molecular weight was calculated as described in (20), where

\[
R = \frac{s_{\text{unknown}}}{s_{\text{standard}}} = \left(\frac{\text{mol wt unknown}}{\text{mol wt standard}}\right)^{1/2}
\]

**TABLE VI**

| Nucleotide used | Reduction after deoxyribonucleotide added |
|-----------------|----------------------------------------|
|                 | None | dATP | dGTP | dCTP | dUTP | dTTP |
| ATP             | 68   | 66   | 40   | 53   | 56   | 38   |
| GTP             | 86   | 58   | 92   | 58   | 52   | 21   |
| TTP             | 50   | 49   | 48   | 54   | 54   | 38   |
| CTP             | 39   | 27   | 38   | 35   | 38   | 25   |
| UTP             | 25   | 20   | 44   | 36   | 25   | 14   |

**Assay conditions are described in the text and in Table III; 2 mM ribonucleotide was used; in the reduction assay 0.1 mM deoxyribonucleotide and in the exchange assay 1 mM deoxyribonucleotide were added.**
Ribonucleotide Reductase from E. gracilis

With rabbit muscle lactate dehydrogenase as a marker. The molecular weight of E. gracilis ribonucleotide reductase was estimated from values given in the literature: rabbit muscle lactate dehydrogenase, 130,000 (24); pig heart malate dehydrogenase, 70,000 (25); pig heart diaphorase, 102,000 (26); L. leichmannii ribonucleotide reductase, 76,000 (27). With these values, the molecular weight of E. gracilis ribonucleotide reductase was estimated to be in the range of 140,000 to 150,000.

**DISCUSSION**

The results presented here clearly indicate that E. gracilis contains a ribonucleoside triphosphate reductase dependent on 5'-deoxyadenosylcobalamin. With the extremely sensitive hydrogen exchange reaction between 5'-deoxyadenosylcobalamin-5'-$\text{H}_2$ and water, low levels of enzyme activity could be detected in cell-free extracts prepared from E. gracilis grown under a variety of conditions. In contrast, extracts of O. malhamensis and O. danica grown under similar conditions are unable to promote this hydrogen exchange. The requirements for a dithiol as a reducing agent, a ribonucleoside triphosphate as a substrate, and 5'-deoxyadenosylcobalamin as a cofactor are identical with those of the reductase system isolated from L. leichmannii. The enzyme from L. leichmannii, the only deoxyadenosylcobalamin-requiring ribonucleotide reductase investigated in detail, shows specific stimulation by addition of deoxyribonucleotides (15, 28), and it has been shown kinetically that the effectors bind to an allosteric site on the enzyme (29). In contrast, the enzyme system from E. gracilis is generally inhibited by addition of deoxyribonucleoside triphosphates, as seen in Table VI. These negative effectors are assumed to bind at a site other than the substrate site, since, in all cases, the reduction of a particular nucleotide is not inhibited by its own product.

Although the ribonucleotide reductase from E. gracilis is similar to that from L. leichmannii in that both have an absolute requirement for deoxyadenosylcobalamin and reduce only the ribonucleoside triphosphates, the E. gracilis enzyme appears to differ from the other reductases so far described in its method of control. Although the precise physiological significance is difficult to assess, it seems logical to assume that during the G phase of the cell division cycle synthesis of deoxyribonucleotides could be maintained at a low level, because ribonucleotide reduction would be inhibited by dTTP and by other deoxyribonucleotides. However, during S phase these deoxyribonucleotides would be required in the synthesis of new DNA and would no longer be available for binding to ribonucleotide reductase, thereby relieving inhibition of this enzyme.

The ribonucleotide reductase of E. gracilis also appears to differ from the other enzymes with respect to molecular weight. The ribonucleotide reductase of E. coli is a large protein consisting of two subunits and a combined molecular weight of 240,000 to 260,000 (19). The enzyme from L. leichmannii has a molecular weight of 76,000 and consists of two subunits (27). The E. gracilis enzyme, with a molecular weight of approximately 145,000, falls between these two. Thus, the ribonucleotide reductase of E. gracilis, with respect to molecular weight and allosteric control, differs markedly from other enzymes which catalyze the reduction of ribonucleotides. It may be that, unlike the more classical metabolic enzymes, ribonucleotide reductase exhibits a high degree of species' individuality.

The earlier observation that E. gracilis grown in the absence of a cobalamin does not respond to deoxyribosides or methionine suggests either that an additional cobalamin-requiring reaction is essential for growth or that the salvage pathway for deoxyribonucleotide synthesis does not play an important role in this organism.

**REFERENCES**

1. Blackley, R. L., *J. Biol. Chem.*, 240, 2173 (1965).
2. Cowles, J., and Evans, H. J., *Arch. Biochem. Biophys.*, 127, 770 (1968).
3. Blackley, H. L., Ghambier, R. K., Nixon, P. F., and Vitols, E., *Biochem. Biophys. Res. Commun.*, 20, 439 (1965).
4. Ahleris, R. H., and Beck, W. S., *J. Biol. Chem.*, 242, 3589 (1967).
5. Richardson, A. W., *Eur. J. Biochem.*, 3, 259 (1968).
6. Hutner, S. H., and Provosoli, L., in S. H. Hutner and A. I. Orloff (Editors), *Biochemistry and physiology of protozoa*, Academic Press, New York, 1955, p. 39.
7. Beck, W. S., Hook, S., and Barnett, B. H., *Biochim. Biophys. Acta*, 55, 455 (1962).
8. Epstein, S. S., Weiss, J. B., Causey, D., and Bush, P., *J. Protozool.*, 9, 336 (1962).
9. Gusevets, I. C., and Barzellel, W. E., in S. P. Colowick and N. O. Kaplan (Editors), *Methods in enzymology*, Vol. III, Academic Press, New York, 1957, p. 941.
10. Gleason, P. K., and Hogenkamp, H. P. C., in D. McCormick and L. Wright (Editors), *Methods in enzymology*, Vol. XVIIIIC, Academic Press, New York, in press.
11. Provosoli, L., *J. Protozool.*, 5, 1 (1958).
12. Eisenstadt, J., and Brawerman, G., in L. Grossman and...
13. Hogenkamp, H. P. C., Ghambar, R. K., Brownson, C., Blakley, R. L., and Vitols, E., *J. Biol. Chem.*, 243, 799 (1968).
14. Bray, G. A., *Anal. Biochem.*, 1, 279 (1960).
15. Blakley, R. L., *J. Biol. Chem.*, 241, 176 (1966).
16. Gornall, A. G., Bardwell, C. J., and David, M. M., *J. Biol. Chem.*, 177, 751 (1949).
17. Bertani, I. E., Haggmark, A., and Reichard, P., *J. Biol. Chem.*, 238, 340 (1963).
18. Blakley, R. L., *Fed. Proc.*, 25, 1633 (1966).
19. Brown, N. C., Canellakis, Z. N., Lundin, B., Reichard, R., and Theolander, L., *Eur. J. Biochem.*, 9, 561 (1969).
20. Martin, R. G., and Ames, B. N., *J. Biol. Chem.*, 236, 1372 (1961).
21. Kornberg, A., in S. P. Colowick and N. O. Kaplan (Editors), *Methods in enzymology*, Vol. 1, Academic Press, New York, 1955, p. 441.
22. Ochoa, S., in S. P. Colowick and N. O. Kaplan (Editors), *Methods in enzymology*, Vol. 1, Academic Press, New York, 1955, p. 735.
23. Mass, V., in W. W. Wood (Editor), *Methods in enzymology*, Vol. 3, Academic Press, New York, 1966, p. 212.
24. Fromm, H. J., *J. Biol. Chem.*, 236, 2688 (1963).
25. Thorne, C., and Kaplan, N., *J. Biol. Chem.*, 238, 1861 (1963).
26. Mass, V., Hoffman, T., and Palmer, G., *J. Biol. Chem.*, 237, 3820 (1962).
27. Panagou, D., Orr, M. D., and Blakley, R. L., *Fed. Proc.*, 29, 915 (1970).
28. Beck, W. S., Goulian, M., Larsson, A., and Reichard, P., *J. Biol. Chem.*, 241, 2177 (1966).
29. Vitols, E., Brownson, C., Gardiner, W., and Blakley, R. L., *J. Biol. Chem.*, 242, 2635 (1967).
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*J. Biol. Chem.* 1970, 245:4894-4899.

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