Evidence for NADH- and NADPH-specific Isozymes of Glutamate Dehydrogenase and the Continuous Inducibility of the NADPH-specific Isozyme throughout the Cell Cycle of the Eucaryote Chlorella*

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SUMMARY

Two isozymes of glutamate dehydrogenase were shown to exist in a thermophilic strain of Chlorella pyrenoidosa. The NADPH:NADH activity ratios of the NADH- and NADPH-specific isozymes were 1:5 and 33:1, and the molecular weights were estimated to be 179,000 and 269,000, respectively. Only the NADH-specific isozyme was detectable in nitrate-cultured cells; the synthesis of the NADPH-specific isozyme was inducible by ammonium.

In light-dark-synchronized cells in nitrate medium, the activity of the NADH-specific isozyme remained constant for 4 hours and then increased continuously for the remainder of the first and into the second synchronous cell cycle.

The induction of the NADPH-specific isozyme was dependent on both RNA and protein synthesis and this isozyme was inducible at all times during the cell cycle. The potential, i.e. maximum rate of induction, increased in a single step during the period of DNA replication and the fold increases in potential and in DNA were essentially equal. These data indicate that the structural gene for this isozyme is continuously available for transcription during the cell cycle of this eucaryotic microorganism, and are consistent with the hypothesis that, under fully induced conditions, the gene dosage of the cell governs the potential.

EXPERIMENTAL PROCEDURE

Organism and Growth Conditions—The thermophilic strain 7-11-05 of C. pyrenoidosa (14) was cultured at 38.5° in glass tubes or in Plexiglas chambers placed in the constant temperature, illuminated water bath described by Hare and Schmidt (15); however, the light intensity was reduced to 550 foot-candles to yield a cell division number of 4. The cultures were bubbled continuously with 4% (v/v) CO2-air.

The cells (28 x 10⁶ per ml) were synchronized, in 2.54-cm diameter glass culture tubes, by three alternating light-dark cycles of 10.8 hours. The synchrony of the cells from the end of the third dark period was improved further by selection of a very uniform population of daughter cells by a modification (16) of the equilibrium density gradient procedure of Sitz et al. (17).

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In synchronous cell cycle studies, the cells (150 to 200 × 10⁶ per ml) were cultured under 550 foot-candles in flat Plexiglas chambers with an internal thickness of 0.80 cm. These cultures were diluted with fresh medium (pre-equilibrated to 38.5° and 4% CO₂-air) one-half hour after initiation of synchronous growth, and then at hourly intervals thereafter. This dilution procedure, a modification of the continuous dilution method of Hare and Schmidt (15), coupled with turbidity measurements at hourly intervals, permitted the culture turbidity to be held essentially constant during synchronous growth.

The composition of the culture medium was described previously (18), except the KH₂PO₄ level was returned to that employed by Sorokin and Myers (19). To give an equivalent amount of nitrogen in both the nitrate- and ammonium-containing media, 0.0225 M KNO₃ or 0.0114 M (NH₄)₂SO₄ was used in the two media with initial pH values of 6.8 and 7.3, respectively. The difference in the initial pH was made because of the tendency of the pH of the nitrate medium and of the ammonium medium to shift upward and downward, respectively, during growth.

Preparation of Cell Material for Analysis—Cells were harvested by centrifugation, washed three times in 0.01 M Tris-HCl buffer (pH 7.7), and resuspended in 0.1 M Tris-HCl buffer (pH 8.25). Cells were maintained at 4° through the harvest and wash procedures. Complete cell breakage was obtained by use of a mechanically driven French pressure cell, model 5-596 (American Instrument Co.), at a pressure of 18,000 to 20,000 p.s.i. Homogenates were assayed directly or supernatants were prepared from these preparations (as described for each figure under "Results"), and then assayed for NADH- and NADPH-glutamate dehydrogenase activity.

Spectrophotometric Assay of Glutamate Dehydrogenase Activity—The spectrophotometric assay was modified from that of Kates and Jones (20). The modifications in the assay solution were as follows: 33.5 mM Tris-HCl buffer (pH 8.25), 300 mM ammonium sulfate, 25 mM α-ketoglutarate, and either 0.265 mM NADH or 0.249 mM NADPH. The oxidation of NADH or NADPH during the assay was measured by the decrease of absorbance at 340 nm by use of a Gilford model 200 recording spectrophotometer. An aliquot of 100 to 500 μl of an extract was placed in a 10-mm path length cuvette, and the decrease in absorbance at 340 nm by use of a Gilford model 200 recording spectrophotometer. An aliquot of 100 to 500 μl of an extract was placed in a 10-mm path length cuvette, and the decrease in absorbance at 340 nm was recorded over a 1- to 2-min interval at a chart speed of 10.3 cm per min, and a sensitivity of 0.05, 0.10, 0.20, or 0.50 A full scale deflection. The reaction rate remained linear over the time interval of assay and was proportional to the amount of enzyme assayed.

One unit of glutamate dehydrogenase activity is defined in the standard way as the amount of enzyme required to oxidize 1 μmole of NADH per min.

Analytical Disc Gel Electrophoresis—The procedure from Davis (21) was employed as described by the Canalco Co. 1 The details of each electrophoresis experiment are described under each set of gel photographs under "Results."

After initial experiments, the Tween solution employed in the standard procedure to coat the walls of the polymerization tubes was omitted to reduce tailing of the NADPH-specific glutamate dehydrogenase isozyme during electrophoresis.

At the completion of electrophoresis, glutamate dehydrogenase activity was detected in the gels with a modified tetrazolium assay (22) solution consisting of the following: 40 ml of 0.1 M Tris-HCl buffer, pH 8.25, 3.0 ml of 1.0 M L-glutamate, pH 7.0; 1.0 ml of 0.0065 M phenazine methosulfate; 2.0 ml of 0.0057 M nitro-blue tetrazolium; 1.3 ml of 0.022 M NAD⁺, or NADPH⁺, or both. The assay solutions containing the gels were incubated in the dark either at 37° for 20 min or at room temperature for 1 hour. The gels were rinsed with distilled water and stored in 7% nacetic acid until photographed.

Elution of Isozymes from Acrylamide Gels—For the experiment in which the isozymes were separated by electrophoresis and then were eluted from the acrylamide gels, the cells were precultured in nitrate medium and then were cultured for 4 hours in ammonium medium in continuous light. The cells were ruptured with the French pressure cell, and 5 ml of homogenate from 10 × 10⁶ cells were centrifuged at 100,000 × g for 1 hour. The supernatant was divided into 12 portions which were loaded onto 12 acrylamide gels. The gels were prepared in tubes, 5 × 150 mm, with 2.0-ml separator gels (7% acrylamide), 0.5-ml stacker gels and sample gels containing 0.1 ml of the supernatant preparation. Elution was performed at 28 μa per tube for 2 hours followed by 3 μa per tube for 1/2 hours.

The isozyme bands were located by incubating one of the set of 12 gels in the tetrazolium assay solution containing both NAD⁺ and NADPH⁺. For elution of the isozymes, the 11 remaining gels were sectioned. Sections of 3 mm containing either isozyme were submerged in 5 ml of 0.1 M Tris-HCl buffer (pH 8.25), and stored at 4° for 30 hours with occasional mixing. The eluted isozymes were separated from the gels by filtration.

Sucrose Density Gradient Centrifugation—Twelve-milliliter linear gradients from 5 to 20% sucrose were prepared (23) from 20% (w/v) sucrose in 0.1 M Tris-HCl buffer, pH 8.25. Enzyme preparations in the same buffer were layered on the gradients with 115 μg of bovine liver catalase as an internal marker. After centrifugation in a Spinco model L ultracentrifuge (SW 40 rotor) at 40,000 rpm for 24 hours (period of maximum speed), the tubes were punctured with a 20-gauge hypodermic needle, 3-drop fractions were collected, and these fractions were assayed for both glutamate dehydrogenase and catalase activities.

Catalase Assay—Catalase activity in gradient fractions was assayed by a method similar to that employed by Martin and Ames (24).

Total DNA, Protein, and Cell Number Measurements—The cells were extracted with 1 N NaOH for 12 hours, neutralized with ice-cold HCl, and precipitated by addition of cold chloroform-acid to a final concentration of 10% (v/v). After centrifugation and two washings with cold 10% chloroform-acid, the precipitate was extracted with 5% trichloroacetic acid for 20 min at 90°, cooled in ice, and centrifuged. The suspensions were stirred frequently during the hot acid extraction procedure. Total DNA in the supernatants was measured by the Burton (25) diphenylamine procedure.

1 Canalco 1969, Research disc electrophoresis instructions, Rockville, Md.
Total protein was determined, by the method of Lowry et al. (26), on the material obtained by extraction of the cells with 1 N NaOH for 12 hours.

Cell number was determined with a model B Coulter counter.

Reagents—Cycloheximide was kindly provided by Dr. G. S. Fonken of Upjohn Co. Actinomyein D was obtained from Schwartz-Mann; α-ketoglutarate, Tris, NAD⁺ (A grade), bovine liver catalase (A grade), Calbiochem, Inc.; NADH and NADPH (Chromato-Pure), P-L Biochemicals, Inc.; L-glutamate and phenazine methosulfate, Mann Research Laboratories; nitro-blue tetrazolium, Sigma Chemical Co.; ammonium sulfate, J. T. Baker Chemical Co.; sucrose (optical grade), Harshaw Chemical Co.; and all reagents for the analytical disc gel electrophoretic procedure (except sucrose), Canaleo Co.

RESULTS

Pattern of Glutamate Dehydrogenase Activity during Cell Cycle of Cells Cultured in Nitrate Medium—The NADH- and NADPH-glutamate dehydrogenase activities per ml of culture remained constant during the first 4 hours of synchronous growth and then increased exponentially into the next cell cycle when the cells were cultured in nitrate medium under continuous light at an intensity which resulted in their division into four daughters (Fig. 1). The NADPH: NADH activity ratio was 1:5 at each hour throughout the cell cycle.

The periodic DNA pattern reported in the present paper differs from the continuous pattern reported (27, 28) earlier for this organism. Analytical biochemistry in the earlier work only made it appear as though DNA accumulated in a continuous fashion during the cell cycle. Although DNA accumulated in a periodic manner, total cellular protein increased continuously and exponentially (Fig. 1).

Induction of NADPH-specific Glutamate Dehydrogenase Isozyme by Ammonium—The 100,000 × g supernatant fractions from cells cultured in either nitrate or ammonium medium were examined for glutamate dehydrogenase activity following analytical disc gel electrophoresis (Fig. 2). A single band of glutamate dehydrogenase activity was observed in the gels after electrophoresis of supernatants from nitrate-cultured cells.

The enzyme with the lower electrophoretic mobility appeared to have high specificity for NAD⁺ than with NADPH. A second, slower moving band of glutamate dehydrogenase activity was detected in supernatants from cells precultured on nitrate and then placed on ammonium medium for 4 hours. The enzyme with the lower electrophoretic mobility appeared to have high specificity for NAD⁺. A 20-fold increase in sample placed on the gels was necessary before the NAD⁺ reactivity of this band was barely detectable. Thus, the disc gel electrophoresis procedure indicated that this species of Chlorella contains only one major isozyme of glutamate dehydrogenase when cultured in the presence of nitrate but has an additional isozyme when cultured in the presence of ammonium.

To determine whether these apparent isozymes were merely an artifact of the disc gel procedure, a comparison was made of the sedimentation profiles in sucrose gradients of the glutamate dehydrogenase activities from nitrate-cultured cells (Fig. 3, A...
FIG. 3. Sedimentation patterns in sucrose density gradients of glutamate dehydrogenase from *Chlorella pyrenoidosa* (strain 7-11-05) cultured in either nitrate or ammonium medium. Light-dark synchronized cells were cultured in glass culture tubes in continuous light at 153 × 10^6 cells per ml. At the 4th hour, 6 × 10^6 cells were harvested from each culture, washed, resuspended (9.0 ml), and ruptured in the French pressure cell as described under “Experimental Procedure.” The resulting homogenates were centrifuged at 100,000 × g for 1 hour, and the supernatants were stored at -20°. From these thawed supernatants, 50-μl aliquots, containing 4.5 or 6.9 mg of protein from the cells of nitrate or ammonium cultures, respectively, were applied on linear gradients of sucrose (5 to 20%, w/v; 12.0 ml) prepared in 0.1 M Tris-HCl buffer (pH 8.25). The gradients were centrifuged at 40,000 rpm (SW 40 rotor) for 24 hours at 3°, a-drop fractions were collected and assayed spectrophotometrically for enzyme activity. Glutamate dehydrogenase units per fraction with NADH (•) and with NADPH (○), and catalase (○—○). A single peak of glutamate dehydrogenase activity was seen from nitrate-cultured cells (Fig. 3A). The NADPH:NADH activity ratio under this peak was 1:5. The sedimentation profile of glutamate dehydrogenase activity, from cells precultured in nitrate and then placed in ammonium medium for 4 hours, showed the peak activity with NADPH to be much greater than the peak activity with NADH and to be placed deeper into the gradient (Fig. 3B). However, the NADH activity still peaked in essentially the same position as shown in Fig. 3A. These results and the data from electrophoresis studies are consistent with the hypothesis that exposure to ammonium induces a second enzyme form which is NADPH-specific.

To estimate the molecular weights of these two isozymes, the mobilities of the two enzyme activities were measured in sucrose density gradients with bovine liver catalase as an internal marker (Fig. 4). Based on the catalase marker, the molecular weights of the NADPH- and NADH-reactive isozymes were calculated (24) to be 269,000 and 179,000, respectively.

Based on the ammonium-induced increase in NADPH-dependent glutamate dehydrogenase activity, while the NADH-dependent activity remained constant, Shatilov et al. (29) have proposed that another strain (Pringsheim, 82T) of *C. pyrenoidosa* also might have two forms of this enzyme.

**Coenzyme Specificities of Glutamate Dehydrogenase Isozymes—** The coenzyme activity ratio for each isozyme was established by separating the isozymes, from ammonium-cultured (4 hours) cells, by analytical disc gel electrophoresis followed by elution from the acrylamide gels as described under “Experimental Procedure.” Spectrophotometric measurements of glutamate dehydrogenase activity with the most reactive coenzyme for each isozyme, indicated that each isozyme eluent contained approximately 38% of the starting activity for that coenzyme. When the eluents containing the separated isozymes were resubjected to electrophoresis, essentially no cross-contamination could be detected (Fig. 5). The NADPH:NADH activity ratio in the eluent for the faster moving isozyme was 1:5, while that for the slower moving isozyme was 33:1.

The separated isozymes were independently subjected to centrifugation, along with a catalase marker, in two identical sucrose density gradients. Inasmuch as the catalase markers moved to corresponding positions in each of the gradients, the data from both gradients are plotted in a single graph (Fig. 6) to facilitate comparison of the activity profiles and mobilities of each isozyme. The fractions from each gradient were analyzed...
FIG. 5. Analytical disc gel electrophoretic patterns of glutamate dehydrogenase isozymes from *Chlorella pyrenoidosa* (strain 7-11-05) before and after their separation and elution in a second disc gel electrophoresis step. Gels were prepared in tubes (5 X 75 mm) using an 0.8-ml separator gel (7% acrylamide), 0.3-ml stacker gel, and a sample gel containing 20 μl of original 100,000 X g supernatants or 100 μl of either isozyme eluent. Electrophoresis was performed in Tris-glycine buffer (pH 8.3) at 2 ma per tube for 2 hours followed by 3 ma per tube for 1 hour. Supernatant activity assayed with NAD+ (A) and NADP+ (B); eluent of the faster moving isozyme assayed with NAD+ (C) and NADP+ (D); and eluent of the slower moving isozyme assayed with NAD+ (E) and NADP+ (F).

FIG. 6. Sedimentation patterns on sucrose density gradients of glutamate dehydrogenase isozymes from *Chlorella pyrenoidosa* (strain 7-11-05), after separation by analytical disc gel electrophoresis, relative to bovine liver catalase. Isozyme eluents (366 μl) were obtained as described under “Experimental Procedure,” and were applied with 115 μg of bovine liver catalase to separate linear gradients of sucrose, centrifuged, and assayed as described in Fig. 5, A and B, and under “Experimental Procedure.” Because the catalase marker in each gradient sedimented to the same position, the data from both gradients are plotted together in this figure. Glutamate dehydrogenase units per fraction with NADH (●), NADPH (○), and catalase activity (●—●—●).

FIG. 7. The relationship between the increase in glutamate dehydrogenase activity and in total cellular protein in *Chlorella pyrenoidosa* (strain 7-11-05) cultured in ammonium medium at different effective light intensities. Light-dark-synchronized cells were placed in continuous light for 4 hours in ammonium medium in glass culture tubes at different cell concentrations to achieve different effective light intensities per cell. At the 4th hour, 2.3 X 10⁶ cells were harvested from each culture, washed, resuspended (6 ml), and ruptured with the French pressure cell as described under “Experimental Procedure.” Total protein and glutamate dehydrogenase activity were determined on 100 μl of the homogenates. Glutamate dehydrogenase activity assayed with NADH (●) and NADPH (○) for cells cultured at 26 X 10⁶, 88 X 10⁶, 237 X 10⁶, and 380 X 10⁶ cells per ml, corresponding to 66 X 10⁻⁹, 50 X 10⁻⁹, 34 X 10⁻⁹, and 26 X 10⁻⁹ mg of protein per cell, respectively.

observed in the supernatant preparations containing the isozyme mixture (Fig. 4). Therefore, the isozyme with an estimated molecular weight of 269,000 and a slower electrophoretic mobility on acrylamide gels will be designated henceforth as the NADPH-specific glutamate dehydrogenase isozyme while the isozyme with an estimated molecular weight of 179,000 and a faster electrophoretic mobility will be designated as the NADH-specific isozyme.

**Dependence of Induction of NADPH-specific Isozyme on Protein and RNA Synthesis**—Molloy and Schmidt (13) have shown total protein accumulation in *Chlorella* to be a function of the effective light intensity per cell. By inoculating cultures at different turbidities or cell concentrations, it is possible to vary the effective light intensity per cell and as a result, to vary the rate of cellular protein accumulation. In the present study, when initial cell concentrations were adjusted to range from 26 X 10⁶ to 380 X 10⁶ cells per ml of culture, the protein level per daughter cell increased inversely with respect to the cell number per ml during a 4-hour growth period in ammonium medium. A linear relationship was observed between the increase in the NADPH-dependent glutamate dehydrogenase activity and the total protein per cell as the growth rate of *Chlorella* daughter cells increased (Fig. 7). In contrast, the NADH-glutamate dehydrogenase activity decreased with increasing protein level per cell over the 4-hour period (Fig. 7). No accumulation of NADH-glutamate dehydrogenase activity occurs during the first 4 hours of the cell cycle for light-dark-synchronized cells (Fig. 1); therefore, the increase in total protein per cell at the lower cell concentrations for glutamate dehydrogenase activity with both coenzymes. However, because of dilution of each isozyme on their respective gradients, the activity of each isozyme could only be detected with the coenzyme of highest reactivity. The recovery of the NADPH and NADH activities from the gradients was 100 and 89%, respectively. The peak activities of each isozyme relative to the catalase peak (Fig. 6) was the same as that previously observed.
Asynchronous cells cultured in nitrate medium were placed in a small Plexiglas chamber in ammonium medium, ammonium medium plus cycloheximide (25 μg per ml), or ammonium medium plus actinomycin D (200 μg per ml) at cell concentrations of 124 × 10⁶ cells per ml and pre-equilibrated (2% CO₂-air; 38.5°) for 15 min in dark. Cultures were then placed in continuous light (550 ft-c) and 3.1 × 10⁶ cells were harvested from each culture at 0, 60, and 80 min. The cells were centrifuged, washed three times in 0.01 M Tris-HCl buffer (pH 7.7), resuspended in 6.0 ml of 0.1 M Tris-HCl buffer (pH 8.3), and ruptured with the French press. The resulting homogenates were centrifuged at 100,000 × g for 1 hour. The glutamate dehydrogenase activity in 500 μl of the supernatant fractions was assayed spectrophotometrically with NADH and with NADPH, and was used to calculate the level of NADPH-specific glutamate dehydrogenase isozyme. A coenzyme activity ratio of 1:5 for the NADH-specific glutamate dehydrogenase isozyme was used in these calculations.

### Table I

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(highest effective light intensities per cell) lowers the specific activity of the NADH-specific isozyme (Fig. 7).

When cycloheximide (25 μg per ml) or actinomycin D (200 μg per ml) was present in ammonium culture medium prior to the onset of an 80-min induction period, the increase in NADPH-glutamate dehydrogenase activity was inhibited relative to the control (Table I). Cycloheximide totally blocked the increase whereas actinomycin D was only 85% effective.

The supernatants from cells cultured under these conditions were subjected to analytical disc gel electrophoresis. The resulting isozyme patterns clearly show the essentially total inhibition of induction of the NADPH-specific isozyme by cycloheximide and the lesser inhibition by actinomycin D (Fig. 8). These data show the dependence of the increased NADPH-glutamate dehydrogenase activity in ammonium-induced cells on both RNA and protein synthesis, and support the inference that the increased activity represents the synthesis of the NADPH-specific glutamate dehydrogenase isozyme rather than activation of a pre-existing enzyme.

Dunn and Schmidt have observed this species of Chlorella to be very impermeable to certain organic compounds. A concentration of 40 μM uridine in the culture medium was required before uridine incorporation into total RNA was constant in a 1-hour incorporation period. A concentration of 200 μg of actinomycin D per ml of culture was required to inhibit this 1-hour incorporation by 50%. However, when actinomycin D was present in the culture medium for 2 hours, uridine incorporation into total RNA was almost totally blocked.

**Measurement of Inducibility and Potential of NADPH-specific Glutamate Dehydrogenase Isozyme during Cell Cycle**—Since the rate of total protein accumulation (13) and the rate of induction of the NADPH-specific glutamate dehydrogenase isozyme (Fig. 7) were shown to be proportional to the effective light intensity per cell, it was necessary, because of the increase in cell volume during synchronous growth, to perform the inductions at a constant culture turbidity rather than at a constant cell number at each hour during the cell cycle. To achieve this end, the parent synchronous culture on nitrate medium, from which the cells were taken for the hourly inductions, was diluted at hourly intervals to maintain a constant culture turbidity. When cells were taken for the hourly inductions, they were washed free of the nitrate medium, resuspended in exactly the same volume of ammonium medium, and placed under the same light intensity in a small Plexiglas culture chamber of internal thickness identical with the parent culture.

The cells from each harvest period were challenged to synthesize the NADPH-specific glutamate dehydrogenase during a 60-min induction period. A visual comparison of the isozyme patterns (in acrylamide gels), from cells in nitrate medium (Fig. 9A) and ammonium-induced cultures (Fig. 9B), clearly shows the NADPH-specific isozyme to be inducible at all stages of the cell cycle, i.e., the NADPH-specific isozyme exhibits continuous...
inducibility during the cell cycle. Since the induction of the NADPH-specific isozyme is dependent upon both protein and RNA synthesis, continuous inducibility strongly suggests that the structural gene of this enzyme is continuously available for transcription throughout the cell cycle. These results are consistent with those of Baechtel et al. (12) who showed continuous inducibility of isocitrate lyase during the cell cycle of the same eucaryote.

If the structural gene of an enzyme is continuously available for transcription, the fully induced rate of synthesis, i.e. potential, of the enzyme should be proportional to the number (dosage) of its structural genes during the cell cycle. Thus, the potential should exhibit the same fold increase as the total DNA, and it should increase during the period of DNA replication.

The induction kinetics of the NADPH-specific isozyme, from 3 different hours of the cell cycle, is shown in Fig. 10. Correction is made for the NADPH activity contributed by the NADH-specific isozyme which remained essentially constant during the 80-min induction periods. The data also was corrected for the culture dilution by the method of Hare and Schmidt (15). A 35-min induction lag, followed by a linear increase in the NADPH-specific isozyme for at least 80 min, was a characteristic of each stage of the cell cycle. Therefore, the slope determined between 35 and 60 min at each hour in the cell cycle was taken as a measure of the potential of the NADPH-specific isozyme at that stage of development.

Since the total induction period (including the 35-min induction lag) for each harvest sample was only 8% of the cell cycle, the potential for each sample was plotted on the cell cycle time scale at a time corresponding to the initiation of each induction period. When the potential from each hour was expressed relative to the average of the first 8 hours, it was observed to increase in a single step during the period of DNA replication (Fig. 11). The fold increases in potential and in DNA were essentially the same, 4.15 and 3.75, respectively.

**DISCUSSION**

Baechtel et al. (12) also observed the potential of isocitrate lyase to remain constant and then to increase in a stepwise manner during the period of DNA replication in this same strain of *C. pyrenoidosa*. The step increases in potential, for both the NADPH-specific glutamate dehydrogenase and isocitrate lyase, occur during the period of DNA replication, suggesting that a direct relationship might exist between the increase in gene dosage and in potential as reported (1) for procaryotes.

The observed difference in the time of initiation of the step increases in potential for these two inducible enzymes might be the result of a difference in the timing of replication of their structural genes during the period of DNA replication. One would expect that in a eucaryotic cell there would be early and late replicating chromosomes or genes. In a synchronous cul-
Mature dehydrogenase isozyme and its relationship to DNA during the cell cycle of Chlorella pyrenoidosa (strain 7-11-05). The cultural conditions and procedures for measuring potential of the NADPH-specific isozyme were identical with those described in Fig. 10. The potential is defined as the maximum rate of induced enzyme synthesis. The initial values for the potential of the NADPH-specific isozyme (○), total DNA (●), and cell number (Δ---Δ) were 8 units per 25 min, 76.3 μg, and 191 × 10^6 cells per ml of culture, respectively.

Nature of eucaryotic cells, even having some degree of randomness, the earliest and latest periods of the interval required for DNA replication should show differential enrichment of these early and late replicating genes. Thus, if the increase in potential in eucaryotic microorganisms is coupled to gene replication, the relationship of these results to those obtained with budding yeast is currently unclear. Although transcription of specific inducible enzymes in budding yeast was reported (8, 9) to be restricted to discrete periods of the cell cycle, when cells were cultured in the continuous presence of inducer, recent evidence indicates that inducible enzymes are continuously available for transcription if inducer is added at periodic intervals during the cell cycle. Studies on the turnover of these inducible enzymes in vivo, DNA patterns, etc., must be performed in the continuous presence of inducer during the cell cycle before this apparent paradox can be resolved. In conclusion, although Tomkins et al. (30) have evidence that an inducible gene is not available for transcription until inducer is added at periodic intervals during the cell cycle, there seems to be no convincing evidence so far to indicate that transcription of specific inducible genes in eucaryotic microorganisms is similarly restricted.

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REFERENCES
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