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Cell Biology

Chitin biosynthesis during *Blastocladiella* zoospore germination: Evidence that the hexosamine biosynthetic pathway is post-translationally activated during cell differentiation*

(post-translational regulation/end product inhibition/cell-wall formation)

C. P. SELITRENNIKOFF, D. ALLIN, AND D. R. SONNEBORN

Department of Zoology, University of Wisconsin, Madison, Wisc. 53706

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ABSTRACT ***De novo*** **construction of a chitinous cell wall accompanies *Blastocladiella emersonii* zoospore germination. At least an order of magnitude increase in *total* hexosamine occurs during germination. This increase is into polymer (chitin) and occurs on schedule in the presence of cycloheximide. Uridine-5′-diphospho-*N*-acetylglucosamine (UDPGlcNAc), both the end product of hexosamine biosynthesis and a substrate for chitin biosynthesis, is a potent inhibitor of the activity of the first pathway-specific enzyme of hexosamine biosynthesis in zoospore extracts. Certain uridine nucleotides, not perceptibly influencing the activity of the first enzyme *per se*, counteract the inhibitory effects of UDPGlcNAc. The concentration of UDPGlcNAc in the zoospore is sufficient to act as an inhibitor of the enzyme, but the amount of UDPGlcNAc is insufficient, by over an order of magnitude, to account for the chitin synthesized during germination. Both the production of UDPGlcNAc and its utilization for chitin synthesis appear to be post-translationally regulated in zoospores and during zoospore germination.**

During the germination phase of the life cycle of the water mold *Blastocladiella emersonii* the nongrowing, motile zoospore rapidly converts to a sessile cell capable of vegetative growth. Accompanying this conversion is the *de novo* construction of a cell wall whose major component by weight is chitin. Morphologically, the cell wall appears on schedule in the presence of cycloheximide at protein-synthesis-inhibiting levels (2–4), suggesting that whatever the controls over initial wall formation are, they do not include a requirement for concomitant protein synthesis.

The terminal enzyme in chitin biosynthesis, chitin synthetase, is present at high specific activity in disrupted zoospore preparations (5, 6) and appears to be preferentially localized in vesicles morphologically characteristic of the zoospore cell type (6). Vesicles fused with the plasma membrane can be observed during the period of initial cell wall formation (7). As previously hypothesized (2, 6, 7), such vesicle–plasma membrane fusions may trigger the construction of the cell wall.

Information presented in this paper indicates that this hypothesis does not furnish a sufficient explanation for the abrupt production of chitin during germination. In particular, the total hexosamine content of the zoospore is found to be insufficient, by at least an order of magnitude, to account for the chitin present after the cell wall is formed. Enzyme activities corresponding to each of the four pathway-specific reactions of hexosamine biosynthesis have now been found in zoospore extracts (6, 8, 9, †). Thus, while the enzymatic machinery to provide substrates for the chitin synthetase reaction is present in the zoospore, the zoospore does not in fact contain sufficient substrates. We report here that: (*a*) cycloheximide does not inhibit the initial, abrupt production of chitin during germination; (*b*) uridine-5′-diphospho-*N*-acetylglucosamine (UDPGlcNAc), a substrate for the synthetase reaction, but also the end product of hexosamine biosynthesis, is a potent negative regulator of the first pathway-specific enzyme of the hexosamine biosynthetic pathway; (*c*) the concentration of UDPGlcNAc in the zoospore is sufficient to act as a feedback regulator of this first enzyme; and (*d*) certain uridine nucleotides, not perceptibly influencing the activity of the first enzyme *per se*, counteract the inhibitory effects of UDPGlcNAc.

MATERIALS AND METHODS

Stock cultures were maintained on standard Cantino peptone–yeast–glucose (PYG) agar and subcultured daily on a 24 hr, 20° life cycle schedule. Freshly released zoospores were harvested by flooding the surfaces of agar cultures with distilled water. Zoospores for some experiments involving hexosamine measurements were derived from liquid cultures grown and sporulated under conditions similar to those previously described (10); the measurements appeared not to be affected by the source of zoospores. Harvested zoospores were immediately filtered through Whatman 541

Abbreviations: UDPGlcNAc, uridine-5′-diphospho-*N*-acetylglucosamine; GlcNAc, *N*-acetylglucosamine; GlcN-6-P, glucosamine 6-phosphate; Fru-6-P, fructose 6-phosphate; cUMP, uridine-3′:5′-cyclic monophosphate; PYG, peptone–yeast–glucose; GS, germination solution.

* This paper is dedicated to Prof. T. M. Sonneborn and is to be included as part of a Festschrift in his honor. Twenty-seven years ago, he published a provocative review article entitled "Beyond the Gene" (1). Near the beginning of the article, he wrote ". . . As has often happened in the history of science, after a general principle is established, exceptions are reported one by one; they are then ignored or assimilated as well as possible into the current pattern of thought until accommodation is so difficult that a revision of the principle is undertaken. . ." In the same article, Sonneborn attempted to relate the available findings from his laboratory to "implications for developmental differentiation." If there is a thread connecting the present article to Sonneborn's "current pattern of thought," it is with respect to this topic. The general principle is the "differential protein synthesis hypothesis." We are aware of no one, including ourselves, who seriously doubts the widespread importance of the principle. However, we report here on what we believe to be an exception to the *sufficiency* of that principle. We attempt no general revision of principle. We do wish to continue to stress, however, that the twin general problems of spatial and temporal localization, which lie at the heart of "developmental differentiation," appear to demand additional "options" than just the acts of transcription and translation *per se*. With this sentiment, we know we are in agreement with Prof. Sonneborn.

† C. P. Selitrennikoff and D. R. Sonneborn, manuscripts submitted.

filter paper to remove sporangial ghosts and cell types other than zoospores. The filtered suspensions were washed with cold distilled water by centrifugation (500 × *g*, 5 min) and the washed pellets were either frozen, lyophilized, and stored in a dessicator at −20° (for enzyme assays and for determination of hexosamine content of zoospores) or diluted into spinner flasks containing germination solution (GS: 50 mM KCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Tris–maleate buffer, pH 6.7) for experiments involving measurements of total hexosamine content during germination. At selected times during germination, samples were withdrawn from the spinner flasks for scoring cell types (7) and for measurement of total hexosamine content. The latter samples were immediately poured into centrifuge bottles containing ice, pelleted, washed once with cold distilled water, and lyophilized. In experiments where samples were harvested at 5 min intervals, the samples were harvested into cold 10% trichloroacetic acid (final concentration), incubated overnight in the cold, centrifuged, and evenly suspended by sonication in a small volume of distilled water.

Total hexosamine was measured on weighed samples (≥10 mg for zoospores; ≥2 mg for samples harvested during germination) that were hydrolyzed in 4 M HCl at 110° in vacuum-sealed tubes for 30 min to 10 hr. The hydrolyzed samples were evaporated to dryness over NaOH and dissolved in 0.01 M HCl. Hexosamine was measured by the procedure of Reissig *et al.* (11). Blanks lacking acetic anhydride were used for each hydrolysate to control for color arising during hydrolysis. For many experiments, crude hydrolysates were applied in 100- to 200-μl volumes to small (about 0.3 ml) Dowex-50 (H^+) preparative columns constructed in disposable pasteur pipettes plugged with glass wool. The columns were washed with 10 column volumes of distilled water and then hexosamines were eluted with 10 column volumes of 0.5 M HCl. The eluates were concentrated, redissolved, and measured as above for crude hydrolysates. Recoveries from the columns, both of authentic glucosamine and of total hexosamine in samples, were >90%. The hexosamines in preparative column eluates from zoospores were further identified by analytical chromatography on a previously calibrated Dowex-50 (H^+) column (1 × 10 cm) as described by Wheat (12), with the exception that the column was developed with 0.2 M HCl rather than 0.3 M HCl. The measurable hexosamine content eluted as a single peak with an R_F identical to authentic glucosamine and clearly separate from other hexosamines. Overall recovery through both the preparative and analytical columns was about 90%.

The types of hexosamine compounds in unhydrolyzed zoospores were examined after quantitative extraction of total hexosamine by the hot water method of Rothman and Cabib (13). The extracts were lyophilized, dissolved in distilled water, and chromatographed on a Dowex-1 (formate) column (1 × 9.7 cm) as described by Molnar *et al.* (14). A linear gradient of ammonium formate (0–0.75 M, pH 7.5; 400 ml total eluate collected in 4-ml fractions) was used.

For enzyme assays, 10 mg of lyophilized zoospores were suspended in 1 ml of 100 mM phosphate buffer, pH 6.8, containing 600 mM sucrose, 1 mM KCl, and 1 mM Na_2 EDTA (buffer A) by pipetting up and down several times with a pasteur pipette. This procedure resulted in complete lysis of zoospores but not of other minor contaminating cell types. The lysates were centrifuged at 10,000 × *g* for 20 min in the cold. To separate small molecules from enzyme activity, the supernatants were applied to 1 × 10.5 cm Sephadex G-25 columns previously equilibrated with buffer A and developed with the same buffer. The first two 750-μl fractions after the void volume were retained for enzyme assays; these fractions contained about 50% of the total enzyme activity and were virtually free (<1%) of added radioactive glutamine, fructose 6-phosphate (Fru-6-P), and UDPGlcNAc. For experiments examining the effects of UDPGlcNAc on enzyme activity, buffer B (addition of 10 mM glutamine to buffer A and readjustment to pH 6.8) was used from the beginning of the procedure, as enzyme activity was partially desensitized to inhibition by UDPGlcNAc when zoospore extracts were prepared in the absence of glutamine.

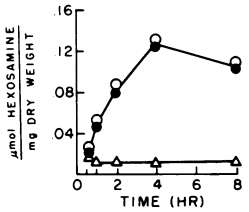

FIG. 1. Hexosamine content as a function of hydrolysis time (4 M HCl, 110°) for zoospores (△), and cells germinated for 14 min in GS (●) or GS containing 1 μg/ml of cycloheximide (○). Freshly released zoospores were obtained from PYG agar cultures, filtered, and washed as described in *Materials and Methods*. One portion of the harvest was lyophilized for total hexosamine determinations; the rest was divided equally for inoculation into the two spinner flask cultures (6 × 10^5 cells per ml). At 14 min, each culture contained about 93% round cells (cell type scoring as in ref. 7). The cultures were harvested into ice, washed, and lyophilized. Weighed amounts of the lyophilized samples were hydrolyzed for the times indicated. Hydrolysates were processed through the preparative column step (see *Materials and Methods*) before measurement of hexosamine content. Recoveries from the columns were >90% for all samples.

L-Glutamine:D-fructose-6-phosphate amidotransferase [formerly EC 2.6.1.16; transferred to EC 5.3.1.19, 2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase (amino-transferring)] activity was assayed in 150-μl reaction mixtures containing various concentrations of substrates and effectors; 50 μl of the above combined column fractions were added to initiate the reactions. The formation of glucosamine 6-phosphate (GlcN-6-P) was determined by the procedure of Ghosh and Roseman (15), with slight modifications as described in detail elsewhere†. Each point in the figures is the average of two determinations (<10% difference between readings). Protein concentration was determined by the method of Lowry *et al.* (16), using bovine serum albumin (fraction V) as a standard. A unit of enzyme activity is the quantity which directed the formation of 1 nmol of GlcN-6-P per min at 25°. Michaelis–Menten constants (K_m) were determined by the standard Lineweaver–Burk graphic method; the inhibition constants (K_i) were determined graphically by the method of Dixon (17).

RESULTS

Hexosamine Content of Zoospores versus Germinating Cells. Measurements of the total hexosamine of zoospores yielded values of 0.05 ± 0.01% (standard deviation) by weight prior to acid hydrolysis and 0.16 ± 0.04% after hydrolysis (eight independent determinations). Maximal values were achieved after mild hydrolysis (1 M HCl, 110°, 30 min), much milder than required to hydrolyze the glycosidic bonds in polymerized hexosamine (Fig. 1 and below). The hexosamine content of zoospores could be quantitatively extracted by the hot water method of Rothman and Cabib

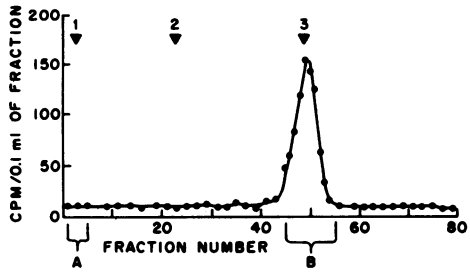

FIG. 2. Characterization of the hexosamine content of zoospores. The hot water extract derived from 59 mg dry weight of zoospores was applied to a Dowex-1 (formate) column precalibrated with authentic glucosamine (GlcNAc) (*1*), GlcN-6-P (*2*), and UDPGlcNAc (*3*). UDP[^{14}C]GlcNAc (●; <0.001 μmol) was included with the zoospore sample analyzed. From 0.285 μmol of total hexosamine (after hydrolysis) applied to the column, 0.075 μmol were recovered in A (fractions 1–5) and 0.200 μmol were recovered in B (fractions 45–55). Assuming 5×10^{-8} mg per zoospore (average value in our laboratory) and a volume of 2.7×10^{-10} ml per zoospore‡, UDPGlcNAc intracellular concentration was calculated to be 6.3×10^{-4} M.

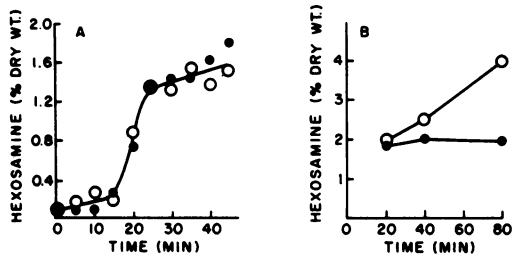

FIG. 3. Total hexosamine levels of zoospores germinating in GS ± cycloheximide. Control spinner flask cultures (○) or cultures containing 2 μg/ml (A) or 1 μg/ml (B) of cycloheximide (●) were inoculated with 10^6 zoospores per ml freshly derived from a liquid growth culture (A) or agar cultures (B). (A) At the indicated times, samples were withdrawn for scoring cell types and for measurements of total hexosamine. Cell type scorings (7) were as follows: 0 and 5 min: 100% zoospores; 10 min: 96–97% zoospores, 3–4% round cells; 15 min: 50% zoospores, 50% round cells; 20 min: 90–95% round cells; 25 min: 98% round cells; 30 min: 85% round cells, 15% germlings (in control; cycloheximide treated cultures do not proceed beyond round cells; refs. 2 and 3); 35 min: 60% round cells, 40% germlings. The samples for hexosamine measurement were withdrawn to cold 10% trichloroacetic acid (final concentration) and processed for crude hydrolysate (4 M HCl, 4 hr, 110°) measurements as described in *Materials and Methods*. In this experiment, the values for the early time points (0–15 min) were not accurately above background readings (blanks lacking acetic anhydride) due to the sample size. (B) 500 ml samples (*ca.* 15 mg dry weight) were harvested into ice and processed through lyophilization as described in *Materials and Methods*. Samples from each time point were hydrolyzed in 4 M HCl, 110° for 4, 6, or 8 hr and processed through preparative Dowex-50 (H^+) columns prior to hexosamine measurement. The points on the curves are derived from the acid hydrolysis times yielding the highest hexosamine measurements.

(13), i.e., measurements of total hexosamine (after acid hydrolysis) in unextracted zoospores and hot water extracts were indistinguishable. About 70% of the extracted hexosamine content consistently cochromatographed with authentic UDP[^{14}C]GlcNAc (Fig. 2) and gave a positive reaction in the Reissig *et al.* (11) hexosamine assay only after mild hydrolysis (1 M HCl, 30 min). This material is UDPGlcNAc, rather than other UDP hexosamines, since only one hexosamine peak was detected by analytical Dowex-50 (H^+) chromatography of hydrolyzed zoospore extracts (recovery ≥90%) and the peak had an R_F identical to authentic glucosamine (see *Materials and Methods*). From three independent quantitations of material cochromatographing with authentic UDPGlcNAc, we estimate the intracellular concentration of UDPGlcNAc to be in the range 1.7 to 6.3×10^{-4} M (‡; Fig. 2 legend).

Upon germination, a rapid increase in total hexosamine was observed; by 20 min of germination, a value of 1.6 ± 0.3% by weight (five determinations) was observed, and by 30 min, a value of 2.5 ± 0.35% (six determinations) was observed. The increased hexosamine content resides virtually entirely in polymerized hexosamine since: (*a*) strong hydrolysis (4 M HCl; 4–8 hr) was required to release the increased levels of hexosamine (Fig. 1); and (*b*) virtually all of the increased hexosamine was recovered in low-speed ($500 \times g$) pellets of sonicated samples (cell-wall-containing fraction) and was KOH-insoluble (chitin). When germination was initiated in the presence of cycloheximide levels known to reversibly inhibit protein synthesis (2, 3), the initial burst in polymerized hexosamine content was observed (Figs. 1 and 3). In several experiments, cycloheximide did appear to inhibit the continued accumulation of polymer beyond a level of about 2% by weight (see, for example, Fig. 3B).

Characterizations of Amidotransferase Activity in Zoospore Extracts. Under the conditions of assay for the amidotransferase, the formation of GlcN-6-P by crude extracts of zoospores was linear with respect to time (through 20 min) and protein concentration (21–81 μg). The reaction was specific with respect to both Fru-6-P and glutamine; neither glucose-6-P nor galactose-6-P effectively substituted for fructose-6-P and asparagine did not substitute for glutamine. The effects of various concentrations of Fru-6-P and glutamine on enzyme reaction velocity are shown in Fig. 4. From these data, the K_m of the crude enzyme for Fru-6-P was calculated to be about 1.6×10^{-3} M and for glutamine to be about 1×10^{-3} M. Even though estimated with crude enzyme, these values are similar to those found with partially purified amidotransferases from several sources§.

B. emersonii zoospore amidotransferase activity is strongly inhibited by UDPGlcNAc. The data in Fig. 5 show that UDPGlcNAc behaves kinetically as a competitive inhibitor [nomenclature of Cleland (25)] with respect to Fru-6-P (calculated K_i, about 5×10^{-6} M) and as an uncompetitive inhibitor with respect to glutamine (calculated K_i, about 7×10^{-5} M). Other UDP sugars were found to be strikingly less inhibitory than UDPGlcNAc (e.g., at saturating substrate concentrations, 0.1 mM UDPGlcNAc, UDPglucose, or UDPgalactose inhibited amidotransferase activity by 72%, 20%, or 10%, respectively). The inhibition of the amido-

‡ The calculations of UDPGlcNAc intracellular concentration are *minimal* estimates for two significant reasons: (*a*) the calculated volume of the zoospore, assuming a sphere of 4 μm radius, is almost certainly a serious overestimate. It is based on two-dimensional diameter measurements (7×9 μm) of cells resting on agar (18). The degree of flatness of such cells seriously influences the accuracy of the volume estimate; (*b*) a significant, but unmeasured, proportion of the volume of the zoospore is taken up by an unusual constellation of membrane-interwoven, singular organelles (mitochondrion, lipid "side body," nuclear cap, nucleus; refs. 6 and 7). It is unlikely that much, if any, of the UDPGlcNAc content of the zoospore resides within this constellation; the enzyme activities involved in the production of UDPGlcNAc, including the amidotransferase, appear to reside outside this constellation†.

§ For example, rat liver (19, 20); rat liver, HeLa, *Bacillus subtilis*, *E. coli* (21); bovine thyroid (22); mung bean (23); and *Neurospora* (24).

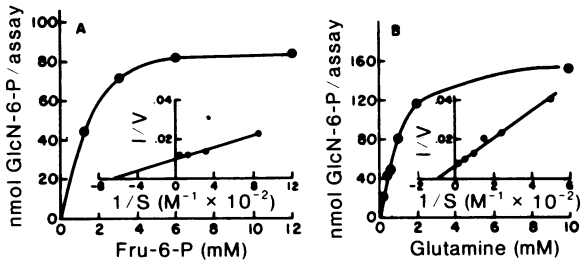

FIG. 4. Effects of various substrate concentrations on amidotransferase activity. Assays were performed under standard conditions (buffer A) at 25°. The amount of GlcN-6-P formed per assay represents the difference between 0 time and 20-min incubations. (A) Each assay contained the indicated Fru-6-P concentration, 10 mM glutamine, 22 μg zoospore extract protein (specific activity; 188 units/mg of protein). The insert is the double reciprocal plot of the data (K_m about 1.6×10^{-3} M). (B) Each assay contained the indicated glutamine concentration, 15 mM Fru-6-P, 45 μg of zoospore extract protein (specific activity; 166 units/mg of protein). The insert is the double reciprocal plot of the data (K_m about 1×10^{-3} M).

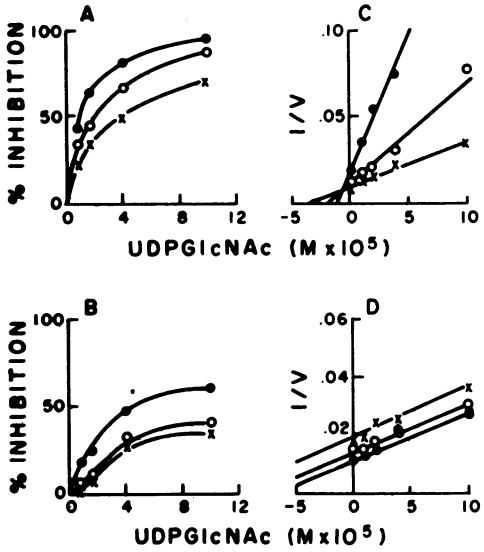

FIG. 5. Effects of various substrate concentrations on inhibition of amidotransferase activity by UDPGlcNAc. Assays were performed under standard conditions using buffer B (in A) or buffer A (in B). The amount of GlcN-6-P formed per assay represents the difference between 0 time and 20-min incubations (over this interval, product formation increased linearly in the presence or absence of UDPGlcNAc). The percent inhibitions of reaction velocities were determined for the indicated UDPGlcNAc concentrations. (A) Percent inhibitions at Fru-6-P concentrations of 1.2 mM (●), 3 mM (○), and 6 mM (×); each assay contained 10 mM glutamine and 29 μg of extract protein (specific activity; 175 units/mg of protein). (B) Percent inhibition of glutamine concentrations of 0.8 mM (●), 2 mM (○), and 4 mM (×); each assay contained 15 mM Fru-6-P and 33 μg of extract protein (specific activity; 133 units/mg of protein). (C) Plot according to Dixon (17) of the data in (A) after reconversion of % inhibition to velocity; competitive inhibition with respect to Fru-6-P, K_i about 5×10^{-6} M. (D) Plot according to Dixon of the data in (B) after reconversion to velocity. To obtain the uncompetitive K_i, the 1/velocity intercepts from a double reciprocal plot of the data were plotted as a function of UDPGlcNAc concentrations (K_i about 7×10^{-5} M).

transferase reaction by UDPGlcNAc is reversible; enzyme activity can be restored by removal of the UDPGlcNAc†.

The effects of uridine nucleotides on zoospore amidotransferase activity were also determined. Under conditions where both substrates were saturating, UTP, UDP, UMP, and 3′:5′-cyclic UMP (cUMP) at concentrations varying from 1 to 5 mM had less than 4% effect on the enzyme reaction (data not shown). However, UTP and UDP had marked stimulatory effects on the UDPGlcNAc-inhibited reaction; the effects of UMP and cUMP were comparatively slight (Fig. 6). These results are similar to those of Winterburn and Phelps (26) using partially purified amidotransferase from rat liver.

DISCUSSION

During the short period of initial cell-wall formation accompanying *B. emersonii* zoospore germination, the total hexosamine level increases at least an order of magnitude; virtually all of the increase is into polymer (chitin). These results indicate that: (*a*) the zoospore does not contain sufficient hexosamine substrates for the chitin synthesized during germination, and (*b*) the block to chitin synthesis is abruptly released during germination. We consider these two points below.

With regard to the first point, we propose that the hexosamine biosynthetic pathway is end-product-inhibited in the zoospore. While high specific activities for each of the four enzymes of the pathway have been found in zoospore extracts (6, 8, 9, †), the end product (UDPGlcNAc) inhibits the activity of *only* the first enzyme†. Inhibition of amidotransferase activity by UDPGlcNAc (Fig. 5; ¶) has been observed with enzyme preparations from several other eukaryotes, but not from bacteria§. The concentration of UDPGlcNAc in the zoospore (see *Results* and ‡) appears to be at least equivalent to that which yields maximal inhibition by UDPGlcNAc of *in vitro* amidotransferase activity (Fig. 5).

Since UDPGlcNAc concentration is also in the range of the apparent K_m of chitin synthetase for diacetylchitobiose formation (5), how is it that the zoospore does not consume the available UDPGlcNAc in the chitin synthetase reaction? Two different, though not mutually exclusive, answers can be offered: (*a*) as mentioned above, there is evidence that chitin synthetase is "packaged" in the zoospore in membrane-bound particles; perhaps *in vivo* substrates are inaccessible to the enzyme within these particles or the enzyme within the particles is in an inactive form (there is precedent for the latter alternative; see ref. 28); (*b*) not only chitin‖ but also *N*-acetylglucosamine (GlcNAc) may be limiting for chitin synthetase activity in the zoospore. Even if all the hexosamine recovered in fraction A, Fig. 2, were GlcNAc (all neutral or positively charged hexosamine derivatives would be recovered in this fraction; see ref. 14), the estimated intracellular concentration would be over an order of magnitude less than the reported (5) apparent K_m of chitin synthetase for GlcNAc (see, however, ‡).

It is most unlikely that the abrupt release of the block to chitin synthesis during germination is controlled in any way by the sudden synthesis of one or more enzymes in the path-

¶ While this paper was being written, K_m and K_i values for *Blastocladiella* zoospore amidotransferase activity were published (27). The reported values are similar to those reported here and are compared in a manuscript submitted†.

‖ If chitin is present in zoospores, it constitutes ≤0.01% by weight. A lower value could not be assigned in the present work because the filtered zoospore preparations were contaminated with 0.1–0.5% germinating cells, as judged by the sensitivity of the cell populations to lysis by detergent [zoospores, but not cell wall-containing, cells are sensitive to detergent lysis (7)].

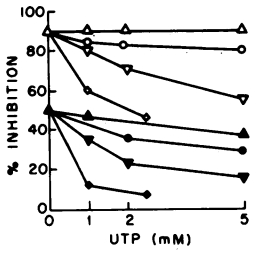

FIG. 6. Effects of uridine nucleotides on UDPGlcNAc-inhibited amidotransferase activity. Assays were performed under standard conditions using buffer B. Reaction mixtures contained 6 mM Fru-6-P, 10 mM glutamine, 25 μg of zoospore extract protein (specific activity; 140 units/mg), either 10^{-5} M (closed symbols) or 10^{-4} M (open symbols) UDPGlcNAc, and the indicated UTP (♦,◊), UDP (▼,▽), UMP (●,○), or cUMP (▲,△) concentrations. The amount of GlcN-6-P formed per assay represents the difference between 0 time and 20-min incubations and the percent inhibition of reaction velocity was computed.

way to chitin synthesis. Not only have high specific activities for all five pathway-specific enzymes been found in zoospore extracts, but also both cell-wall formation (2, 3) and initial chitin production (this paper) occur on schedule in the presence of cycloheximide. Part of the release mechanism may indeed involve activation of the chitin synthetase reaction upon fusion of vesicles with the plasma membrane. Vesicle–plasma membrane fusion as a means of bringing molecules involved in cell-wall construction to the cell surface appears to be a general phenomenon; documentations have been widely reported using a variety of eukaryotic microorganisms and higher plants. Nevertheless, activation of the chitin synthetase reaction *per se* does not solve the problem of providing an order of magnitude increase in total hexosamine. The flux of metabolites in the pathway to UDPGlcNAc *must* undergo an abrupt, radical increase. This part of the release mechanism is likely to involve relief from inhibition of activity of the first enzyme in the pathway. Lowering of the UDPGlcNAc concentration, perhaps "pulled" by the chitin synthetase reaction, is a possibility. In addition, other small molecular weight compounds, particularly when assayed in the presence of UDPGlcNAc, affect the activity of the first enzyme in zoospore extracts†. For example, uridine nucleotides, especially UTP and UDP, counteract the inhibitory effects of UDPGlcNAc (Fig. 6). Thus, an elevation of UTP concentration could have the same effect on relieving inhibition of the first enzyme as a depression in UDPGlcNAc concentration.

Post-translational regulation of biosynthetic pathways by end-product inhibition is, of course, a well documented general phenomenon. It has typically been interpreted in the context of physiological (metabolic) adaptation to changing cellular environments. The evidence discussed in this paper suggests that this type of regulation also operates as part of a normal developmental sequence. Hexosamine and chitin biosyntheses are "turned off" in the zoospore; their rapid "turn on" during germination accompanies the developmental transition from a phase where no cell wall is present to a phase where a chitin-containing wall is present. It is not inconceivable that in other developmental situations where very rapid changes in biosynthesis are called for, a similar type of regulation might be operative. It is worth underscoring that, for end-product inhibition of enzyme activity to be operative in a developmental sense, it would appear that *utilization* of the end product must also be subject to coordinated developmental control. Such appears to be the case in *B. emersonii* zoospores and during zoospore germination.

1. Sonneborn, T. M. (1948) *Am. Sci.* **37**, 33–59.
2. Soll, D. R. & Sonneborn, D. R. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 459–463.
3. Lovett, J. S. (1968) *J. Bacteriol.* **96**, 962–969.
4. Silverman, P. M., Huh, M. M. & Sun, L. (1974) *Dev. Biol.* **40**, 59–70.
5. Camargo, E. P., Dietrich, C. P., Sonneborn, D. R. & Strominger, J. L. (1967) *J. Biol. Chem.* **242**, 3121–3128.
6. Myers, R. B. & Cantino, E. C. (1974) *"The gamma particle," Monographs in Developmental Biology*, ed. Wolsky, A. (S. Karger, Basel, Switzerland), Vol. 8, 117 pp.
7. Soll, D. R., Bromberg, R. & Sonneborn, D. R. (1969) *Dev. Biol.* **20**, 183–217.
8. Giddings, T. H. & Cantino, E. C. (1974) *J. Bacteriol.* **120**, 976–979.
9. Normann, J., Myers, R. B., Giddings, T. H. & Cantino, E. C. (1974) *Biochim. Biophys. Acta* **302**, 173–177.
10. Lodi, W. R. & Sonneborn, D. R. (1974) *J. Bacteriol.* **117**, 1035–1042.
11. Reissig, J. L., Strominger, J. L. & Leloir, L. F. (1955) *J. Biol. Chem.* **217**, 959–966.
12. Wheat, R. W. (1966) in *Methods in Enzymology*, eds. Neufeld, E. F. & Ginsberg, V. (Academic Press, New York), Vol. 8, pp. 72–73.
13. Rothman, L. B. & Cabib, E. (1969) *Biochemistry* **8**, 3332–3341.
14. Molnar, J., Chuo, H. & Ikahara, Y. (1971) *Biochim. Biophys. Acta* **239**, 401–410.
15. Ghosh, S. & Roseman, S. (1962) in *Methods in Enzymology*, eds. Colowick, S. P. & Kaplan, N. O. (Academic Press, New York), Vol. 5, pp. 414–417.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
17. Dixon, M. (1953) *Biochem. J.* **55**, 170–171.
18. Cantino, E. C. & Hyatt, M. T. (1955) *Antonie van Leeuwenhoek* **19**, 25–70.
19. Miyagi, T. & Tsuiki, S. (1971) *Biochim. Biophys. Acta* **250**, 51–62.
20 Winterburn, P. J. & Phelps, C. F. (1971) *Biochem. J.* **121**, 701–709, 711–720, 721–730.
21. Kornfeld, R. (1967) *J. Biol. Chem.* **242**, 3135–3141.
22. Trujillo, J. L. & Gan, J. C. (1974) *Int. J. Biochem.* **5**, 515–523.
23. Vessal, M. & Hazzid, W. J. (1972) *Plant Physiol.* **49**, 977–981.
24. Endo, A., Kakiki, K. & Misato, T. (1970) *J. Bacteriol.* **103**, 588–594.
25. Cleland, W. W. (1963) *Biochim. Biophys. Acta* **67**, 104–137.
26. Winterburn, P. J. & Phelps, C. F. (1971) *Biochem. J.* **121**, 711–720.
27. Normann, J., Giddings, T. H. & Cantino, E. C. (1975) *Phytochem.* **14**, 1271–1274.
28. Cabib, E., Ulane, R. & Bowers, B. (1974) in *Current Topics in Cellular Regulation*, eds. Horecker, B. L. & Stadtman, E. R. (Academic Press, New York), Vol. 8, pp. 1–32.