Regulation of the hexosamine biosynthetic pathway in the water mold *Blastocladiella emersonii*: Sensitivity to endproduct inhibition is dependent upon the life cycle phase

(enzyme regulation/feedback inhibition/metabolic regulation)

CLAUDE P. SELITRENNIKOFF*, NORMAN E. DALLEY†, AND DAVID R. SONNEBORN†

*Department of Anatomy, University of Colorado Health Science Center, 4200 East Ninth Avenue, Denver, Colorado 80262; and †Zoology Research Building, 1117 West Johnson Street, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT Chitin, a homopolymer of *N*-acetylglucosamine (GlcNAc), is the major macromolecular constituent of *Blastocladiella emersonii* cell walls. Zoospores do not possess a wall nor do they contain sufficient total hexosamine to account for the chitin content of the wall abruptly formed during germination. UDPGlcNAc, both the endproduct of hexosamine biosynthesis and the substrate for chitin synthesis, is present in zoospores in sufficient concentration to inhibit the first hexosamine pathway-specific enzyme activity. Net chitin accumulates in register with dry weight during exponential growth, but does not accumulate appreciably during the succeeding sporulation phase. Predicted relationships among net rates of chitin synthesis, UDPGlcNAc concentrations, and UDP plus UTP concentrations throughout the life cycle are explored, as are the assumptions upon which the predictions were based. We find that the sensitivity of the first hexosamine pathway-specific enzyme to endproduct inhibition is *not* constant throughout the life cycle; sensitivity is very high in the zoospore phase, decreases dramatically during germination, remains very low through the growth phase, and increases gradually to the zoospore level during sporulation. The organism appears to have evolved endproduct regulation in this case as an adaptation to “hard-times” phases of the life cycle—i.e., as a safeguard against overproduction of end product (UDPGlcNAc) when its utilization in cell wall (specifically chitin) synthesis is curtailed. Conversely, the organism effectively discards this mode of regulation during “good times,” when the demands for end product are evidently greater than endproduct inhibition would otherwise permit.

During germination in the water mold *Blastocladiella emersonii*, entire cell populations quickly (within minutes) convert from a cell type lacking a cell wall (zoospore) to one possessing a cell wall (round cell, or cyst). This *de novo* formation of cell wall is accompanied by a sudden accumulation of chitin (1), the predominant macromolecule of *B. emersonii* cell walls (2). Round cells convert to germlings, which are then capable of coenocytic growth. During the exponential phase of growth, net chitin synthesis keeps pace with increase in dry weight (see below). The sporulation phase, elicited in the laboratory after removal of nutrients, includes the conversion of multinucleate sporangia into populations of uninucleate, wall-less zoospores which are then released from each sporangium, leaving behind the sporangial cell wall (see ref. 3 for review).

The abrupt formation of the initial cell wall, as well as the net synthesis of chitin that accompanies it, occur on schedule in the presence of concentrations of cycloheximide that inhibit protein synthesis (1). At least under our laboratory conditions, zoospores do not contain enough substrate, uridine-5'-diphospho-*N*-acetylglucosamine (UDPGlcNAc), by over an order of

magnitude, to account for the chitin synthesized during this period. Zoospore extracts contain high levels of the four enzyme activities of the hexosamine biosynthetic pathway, of which UDPGlcNAc is the end product. UDPGlcNAc specifically inhibits only the first pathway-specific enzyme activity and does so at concentrations that have been estimated to occur in the zoospore. We have interpreted these and other results to indicate that the hexosamine pathway is effectively endproduct inhibited in the zoospore and that during germination, abrupt relief from inhibition is metabolically coupled with activation of chitin synthesis (1, 4).

In zoospore enzyme preparations, UDPGlcNAc behaves kinetically as a linear competitive inhibitor against one of the substrates (fructose-6-phosphate) of the first hexosamine pathway-specific enzyme [the amidotransferase; 2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase (amino-transferring), EC 5.3.1.9] (1, 4, 5). In addition, to inhibit enzyme activity, UDPGlcNAc evidently must interact with the enzyme at a nonsubstrate site at which uridine nucleoside phosphates (UTP, UDP > UMP) and UDPGlcNAc mutually exclude one another (4). This is of particular interest in that utilization of UDPGlcNAc in chitin synthesis results in the production of UDP. Together with other information, this led us to formulate a series of predictions concerning changing levels of UDPGlcNAc and of the ratio UDPGlc/(UDP + UTP) throughout the life cycle (4). Briefly, the predictions were that: (i) both UDPGlcNAc concentration and the ratio would be highest in the zoospore, certainly high enough to effectively “shut down” the hexosamine pathway; (ii) at least the ratio would decrease abruptly during the initial burst of chitin synthesis; (iii) both variables would be lowest throughout the growth phase; and (iv) both variables would return to zoospore levels during the sporulation phase. These predictions relied upon certain simplifying assumptions (4); both the predictions and the assumptions are explored in this paper. One of the assumptions turns out to be invalid; the *sensitivity* of amidotransferase to feedback inhibition of activity does not remain constant throughout the life cycle. The life cycle pattern of sensitivity is interpreted in a broader biological context (see *Discussion*).

MATERIALS AND METHODS

Materials. Polyethyleneimine-cellulose plates (Brinkman) were washed with 10% (wt/vol) NaCl and methanol and stored in the dark at 4°C. $H_3^{32}PO_4$ was obtained through the Uni-

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Abbreviations: GlcNAc, *N*-acetylglucosamine; UDPGlcNAc, uridine-5'-diphospho-*N*-acetylglucosamine; amidotransferase, 2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase (amino-transferring), EC 5.3.1.19.

versity of Wisconsin Radiopharmacy Service. UDP-[U-¹⁴C]-GlcNAc was obtained from Amersham. Other chemicals were obtained as reported (4).

Determinations of Dry Weight, Chitin, and Specific Enzyme Activities. Zoospore inocula were prepared, grown, and sporulated in a New Brunswick Microferm fermentor as described (6), with 1 mg of dextran sulfate per ml (Pharmacia) added to the growth medium. For dry weight determinations, 2-liter samples were removed at selected times and filtered over weighed Millipore filters which were then dried to constant weight and weighed.

For enzyme assays and chitin determinations, 1- to 3-liter samples were washed twice by centrifugation ($500 \times g$, 5 min) in ice-cold distilled water, and the pellets were resuspended in ice-cold buffer appropriate for each enzyme. The suspensions were disrupted by sonication (6) and centrifuged at 4°C ($10,000 \times g$ for 20 min or $27,000 \times g$ for 10 min). For chitin determinations, the pellets were frozen and stored at -20°C. For chitin synthetase (UDP-2-acetamido-2-deoxy-D-glucose:chitin 4- β -acetamidodeoxy-D-glucosyltransferase, EC 2.4.1.16) assays, either the $10,000 \times g$ pellets were resuspended and subjected to a low-speed centrifugation ($220 \times g$, 4 min) to remove whole cells and cell debris (one experiment) or the low-speed supernatants were used directly without a prior $10,000 \times g$ centrifugation (two experiments). For assays of the hexosamine pathway enzymes, the $10,000 \times g$ supernatants were passed through equilibrated Sephadex G-25 columns as described (4) in order to separate enzyme activities from endogenous substrates and other small molecular weight effectors.

For chitin determinations, the frozen pellets were thawed, suspended in distilled water, and disrupted by sonication. The pellets obtained after centrifugation at $10,000 \times g$ for 10 min were suspended in 4 M HCl and hydrolyzed under reduced pressure for 4 hr at 110°C (1). The samples were lyophilized twice to remove HCl, resuspended in distilled water, and centrifuged at $10,000 \times g$ for 4 min. Aliquots of the supernatants were assayed for hexosamine as described (7).

Enzyme assays were performed as described (4), except that the buffer for chitin synthetase assays was reduced to 0.375 μ mol of Tris-HCl and 0.150 μ mol of UDP-[U-¹⁴C]GlcNAc (0.17 Ci/mol; 1 Ci = 3.7×10^{10} becquerels) was used as substrate. All enzyme assays were done in duplicate using three incubation times and were performed within less than 1 hr after cells were harvested. Units of enzyme activity were nmol of product formed per min at 25°C. Specific enzyme activities were calculated as units/mg of protein after protein concentrations were determined by either the method of Lowry *et al.* (8) or the method of Bradford (9).

Metabolite Determinations. Dish cultures were grown and sporulated under standard conditions (10) except that the growth medium was supplemented with 10 μ g of streptomycin sulfate and 400 units of penicillin G per ml. $H_3^{32}PO_4$ (2 μ Ci/ml; final specific activity, 20 Ci/mol) was present in both the growth medium and the sporulation solution, to which 100 μ M sodium phosphate buffer (pH 6.8) was added to keep the specific radioactivity constant. Sporulation was at 33°C for 4 hr. The resulting zoospore populations were harvested and a portion was filtered, washed, and quick-frozen. The remainder was sent through a second life cycle either under the same conditions as for the previous life cycle except that both growth (6 hr) and sporulation were at 27°C or under suspension-culture conditions [spinner-flask cultures (10) contained 10^8 cells in 1 liter of growth medium (6) supplemented with 1 mg of dextran sulfate per ml, antibiotics, and $H_3^{32}PO_4$ as above; sporulation conditions were as described (10) with the same sporulation solution as above]. At selected times during the second life cycle, 50- to

100-ml samples were removed from the suspension cultures or three to five entire dish cultures were harvested after the dish bottoms were scraped with a rubber policeman. The samples were washed by centrifugation ($500 \times g$ for 2 min at 4°C), quick-frozen, and lyophilized in Nalgene centrifuge tubes. The samples were each suspended in 2 ml of ice-cold 6% (vol/vol) formic acid, disrupted by sonication, and centrifuged at $16,000 \times g$ for 20 min at 0°C. Pellets were re-extracted with 1 ml of 6% formic acid. The final pellets were saved for protein determinations. The two supernatants were pooled, frozen, and lyophilized in Falcon plastic tubes.

The lyophilized formic acid extracts were resuspended in 50–100 μ l of 50 mM MgCl₂/25 mM Tris-HCl, pH 7.4, and 10–20 μ l was applied to the origin of polyethyleneimine-cellulose plates along with 10 μ g each of nonradioactive UMP, UDP, UTP, and UDPGlcNAc standards. The plates were immersed in methanol, air dried, and subjected to two-dimensional chromatography (11). The spots corresponding to each standard were identified by ultraviolet light; they were cut out and radioactivity was measured by liquid scintillation. In control experiments, the material from each spot was rerun in at least two separate solvent systems; in each case, only one spot of radioactivity was found that corresponded exactly to the position of the authentic, unlabeled standard. In other experiments, recoveries were estimated after ¹⁴C-labeled authentic standards were added to unlabeled zoospore extracts. The data are corrected both for recovery and for decay of ³²PO₄³⁻.

Calculations. Radioactivity determinations of metabolite were converted to intracellular metabolite concentrations, assuming random distribution, on the basis of the following volume estimates. The zoospore was assumed to be a sphere with a radius of 4 μ m and thus a volume of 2.7×10^{-10} ml (1). Zoospores contained 20 ± 5 pg of Lowry protein per cell. The volume of other cell types was estimated by using the relationship 1 μ g of Lowry protein per 0.013 μ l of cell volume, as determined for the zoospore.

The level of functioning of zoospore amidotransferase (ν/V_{max}) was calculated from equation 4 in ref. 4, expanded to include the concentrations and kinetic constants for each of the three uridine nucleoside phosphates (4) and the metabolite concentrations estimated in this paper and assuming that fructose-6-phosphate was present at K_m levels.

RESULTS

Net Chitin Synthesis During the Life Cycle. We previously reported (1) that zoospores do not contain chitin ($\leq 0.01\%$ of the dry weight), but that during germination chitin accounts for about 1.6% and 2.5% of the dry weight by 20 min and 30 min, respectively. The data in Fig. 1 indicate that throughout the exponential growth period examined (2–6 hr at 27°C), net chitin synthesis keeps pace with increase in dry weight (mass doubling time in each case, ≈ 1.3 hr) and, if net chitin increases at all during sporulation, the increase is slight ($<13\%$). The slight drops in apparent chitin content observed between the last time point from the growth phase (6 hr) and the first time point of sporulation (1 hr) are probably due to alterations in cell concentration accompanying the procedures used to initiate sporulation.

Metabolite Levels During the Life Cycle. To examine the predictions concerning changing levels of UDPGlcNAc and the uridine nucleoside phosphates throughout the life cycle, we wished to achieve and then maintain constant specific radioactivities of these metabolites by an *in vivo* radiolabeling procedure. Cultures were prelabeled for an entire life cycle in the presence of the same (nonlimiting) concentration and specific radioactivity of $H_3^{32}PO_4$ as used during a succeeding

FIG. 1. Chitin content (●, ○) and dry weight (Δ) during the life cycle. Zoospores ($10^9/14$ liters) were grown and sporulated, and chitin content (as GlcNAc) and dry weight were determined. ○ and ●, Two independent experiments. Δ, Each point represents an independent determination from one experiment.

life cycle. The radioactivity of each metabolite was measured and converted to metabolite concentration (see Table 1). The data of Table 1 indicate that maximal radiolabeling was achieved by the end of the first life cycle ("input" zoospores) and maintained through the second life cycle ("output" zoospores)—i.e., within experimental error, the metabolite concentrations calculated from radioactivity measurements were the same for input and output zoospores.

Table 1 is of interest for two further points. First, the values determined for UDPGlcNAc concentration independently confirm those obtained by colorimetric determinations after column chromatography of zoospore extracts (1) and indicate that zoospore UDPGlcNAc concentration, assuming random distribution,[†] is some 2 orders of magnitude above the apparent K_i UDPGlcNAc (5 μ M) with respect to zoospore amidotransferase activity. The second point is that the zoospore metabolite concentrations can be used to provide an estimate (probably maximal[†]) of the level of functioning of the amidotransferase (v/V_{max}) of about 1.7%, thus further indicating that amidotransferase activity, and hence the hexosamine biosynthetic pathway, is indeed effectively endproduct-inhibited in the zoospore.

The determinations of metabolite levels during the life cycle (second labeling cycle) are plotted in Fig. 2. Note that the levels of each metabolite are *lowest* in the zoospore, *increase* during the growth phase, and decrease during sporulation. The "overshoot" in UDPGlcNAc concentration during early sporulation is noteworthy (see *Discussion*); over the same time interval, slight decreases in uridine nucleotide phosphate levels, where they occur, obviously do not compensate quantitatively for the increased UDPGlcNAc levels. Conversely, during later phases of sporulation (beyond 2 hr), sharp decreases in UDPGlcNAc levels are not accompanied by corresponding increases in uri-

Table 1. Intracellular concentrations of metabolites in zoospores*

Metabolite	Intracellular concentrations $\pm$ SD, μ M			
	Input zoospores	Output zoospores		
		Dish	Spinner flask	Avg.
UTP	90 $\pm$ 50	115 $\pm$ 10	45	92 $\pm$ 40
UDP	50 $\pm$ 10	55 $\pm$ 20	60	57 $\pm$ 10
UMP	210 $\pm$ 25	210 $\pm$ 45	240	220 $\pm$ 40
UDPGlcNAc	400 $\pm$ 125	510 $\pm$ 280	320	447 $\pm$ 220

* Input zoospores were from dish cultures ($n = 5$) radiolabeled with $H_3^{32}PO_4$ for one entire life cycle. Output zoospores were from cultures additionally radiolabeled throughout a second life cycle, either under dish culture ($n = 2$) or spinner-flask culture ($n = 1$) conditions. Intracellular concentrations were calculated by assuming random distribution and complete radiolabeling to the same specific radioactivity as in the medium, knowing the number of zoospores per sample, and using the zoospore volume estimate in *Materials and Methods*.

dine nucleoside phosphate levels. Changes in UMP, UDP, and UTP levels throughout the life cycle are evidently not quantitatively coordinated, but the levels of UMP predominate over those of UDP and UTP (Table 1 and Fig. 2).

The data of Fig. 2 do not agree with the previously predicted life cycle pattern of changes in metabolite levels (see Introduction). However, for technical reasons, the change predicted to take place during the abrupt initial formation of cell wall accompanying zoospore germination was not examined. Our failure to confirm other predictions led us to examine the assumptions upon which the predictions were based.

Life Cycle Patterns of Enzyme Activities. One of the assumptions was that amidotransferase is the rate-limiting enzyme activity for the hexosamine pathway throughout the life cycle, as was previously reported to be the case for zoospores (4). The life cycle patterns for each of the four pathway-specific enzyme activities were determined with cell extracts from which endogenous metabolites had been removed prior to assay (see *Materials and Methods* and ref. 4). The data are presented in terms of percent maximal specific activity for each enzyme

FIG. 2. Intracellular concentrations of metabolites throughout the life cycle. Input zoospores, derived as in Table 1, were added either to dish cultures (two complete experiments) or to spinner-flask cultures (three experiments, three time points each) containing $H_3^{32}PO_4$. At the indicated times, culture samples were collected and processed. Radioactivities were converted to concentrations of metabolites. Individual points represent mean values from at least three experiments; SD are indicated. □, UTP; ○, UDP; Δ, UMP; ●, UDPGlcNAc.

[†] Because of the unusual structure of the zoospore, the effective concentrations of the metabolites are probably somewhat higher than the estimates based on random distribution. A significant proportion of the zoospore volume is taken up by an unusual constellation of membrane-interwoven, singular organelles (see ref. 3 for review). Evidence exists (4) that the hexosamine biosynthetic pathway enzymes are located outside this constellation, as probably are the metabolites of concern. An estimate of zoospore water volume, rather than cell volume, has recently been provided (12); if that estimate were used, the metabolite concentrations would be approximately tripled. Thus, the calculation of the level of functioning of the hexosamine pathway in the zoospore is probably an overestimate.

FIG. 3. Life cycle patterns of enzyme activities. Zoospores ($0.5\text{--}2 \times 10^8/\text{liter}$) were grown and sporulated. Specific enzyme activities were determined and plotted as percentages relative to the highest specific activity (100%) for each enzyme. At least two separate life cycle experiments were performed for each enzyme activity. Enzyme activities: ●, amidotransferase; ▲, glucosamine-phosphate acetyltransferase (EC 2.3.1.4); ○, acetylglucosamine phosphomutase (EC 2.7.5.2); □, chitin synthetase (EC 2.7.7.23). The zoospore (100%) specific enzyme activities were (in nmol/mg of protein per min): amidotransferase, 55; glucose-phosphate acetyltransferase, 227; acetylglucosamine phosphomutase, 190; and uridylyltransferase, 110. The 100% specific enzyme activity for chitin synthetase (6-hr point) was 29 nmol/mg of protein per min. ■, The plot for amidotransferase was recalculated using the values for percent inhibition in the presence of $500 \mu\text{M}$ UDPGlcNAc presented in Fig. 4.

(Fig. 3). In each case, the range of variation of enzyme activity throughout the life cycle was fairly small (≈ 3 -fold) and the same general pattern was observed—i.e., relatively high activities in zoospores, relatively low activities during the growth phase, and return to the high activities by the end of sporulation. Comparisons of the specific enzyme activities obtained indicate that amidotransferase activity is indeed the rate-limiting activity throughout the life cycle.

Two additional plots are included in Fig. 3 which are, in general, “mirror images” of the life cycle pattern just discussed. When endproduct inhibition is taken into account (see below), the life cycle patterns for both amidotransferase activity (Fig. 3, ■—■) and for chitin synthetase activity[§] (Fig. 3, □—□) generally conform to the life cycle pattern for rates of net chitin accumulation (see Fig. 1)—i.e., very low in zoospores, high and roughly constant during growth, and returning to zoospore levels during sporulation.

[§] Chitin synthetases from certain fungi can be recovered in predominantly protease-activatable zymogenic form (see ref. 13 for review), which obviously could influence the life cycle pattern of chitin synthetase activity. However, *Blastocladiella* chitin synthetase exhibits relatively high activity prior to protease treatment and relatively weak protease “activation” followed by extreme sensitivity to protease inactivation (unpublished data) similar to that reported with the mycelial, as contrasted with the yeast, form from *Mucor* (ref. 14, but see ref. 15) and with the mycelial form from *Allomyces* (15), the latter a close relative of *Blastocladiella*. In any case, during those phases of the life cycle when chitin synthesis is low at best (sporulation phase) or not detected (zoospore phase), the *in vitro* values for chitin synthetase activity obviously do not accurately reflect the *in vivo* situation. This has been recognized previously (1, 4, 16); the precise explanation is yet to be documented.

Sensitivity of Amidotransferase to Endproduct Inhibition Throughout the Life Cycle. Another assumption was that the kinetic parameters of amidotransferase activity do not change significantly throughout the life cycle. The assumption is apparently valid with respect to several parameters—substrate specificity, K_m values, V_{max} , and pH profile of activity. However, it is invalid with respect to another parameter—sensitivity to endproduct (UDPGlcNAc) inhibition. Under the conditions of the experiments summarized in Fig. 4, zoospore enzyme activity was inhibited $79 \pm 7\%$ and $93.5 \pm 3\%$ in the presence of $100 \mu\text{M}$ and $500 \mu\text{M}$ UDPGlcNAc, respectively (10 experiments). By 1 hr after inoculation into growth medium (shortly after the completion of germination), cell samples displayed only weakly inhibitable enzyme activity ($9.5 \pm 4\%$ and $16.5 \pm 6.5\%$ at $100 \mu\text{M}$ and $500 \mu\text{M}$ UDPGlcNAc, respectively; five experiments). Cell populations sampled throughout the growth phase displayed enzyme activities that were not inhibitable above background, even at $500 \mu\text{M}$ UDPGlcNAc. More detailed kinetic experiments have indicated that the apparent K_i UDPGlcNAc is some 2 orders of magnitude higher for growing cell than for zoospore enzyme activity. Note in particular (Fig. 4) that inhibitable enzyme activity returns only gradually and roughly linearly during sporulation.

DISCUSSION

B. emersonii displays a feedback-inhibitable metabolic pathway during a portion of its life cycle only to effectively discard this mode of regulation during other phases of its life cycle. Our working hypothesis is that the organism evolved feedback inhibibility in this case as an adaptation to “hard times,” as contrasted with the “classical” prokaryotic situation in which effective inhibition of feedback-inhibitable biosynthetic pathways generally operates during “good times” (i.e., when appropriate metabolites are supplied in ample amounts in the nutrient environment). In *B. emersonii*, during good times, when growth and chitin (cell wall) synthesis are occurring, the requirements for end product (UDPGlcNAc) are evidently greater than endproduct inhibition would otherwise permit. The inhibitable amidotransferase apparent K_i with respect to UDPGlcNAc is $\approx 5 \mu\text{M}$ (1, 4, 5), whereas the chitin synthetase

FIG. 4. Life cycle pattern of sensitivity of amidotransferase activity to endproduct inhibition. Enzyme activity was measured at each time point in the presence of $0 \mu\text{M}$, $100 \mu\text{M}$ (●), and $500 \mu\text{M}$ (▲) UDPGlcNAc and percent inhibitions of enzyme activity in the presence of the two concentrations of UDPGlcNAc were separately calculated. Growth and sporulation conditions were as described in Fig. 3. The zero-time and 1-hr points are averages from 10 and 5 experiments, respectively. The remaining time points are from two independent, complete life cycle experiments. ▲, The level of inhibition during the growth phase (2–6 hr) was too low to be reliably determined, but was certainly less than 10% even in the presence of the higher concentration of UDPGlcNAc ($500 \mu\text{M}$).

apparent K_m with respect to UDPGlcNAc is $\approx 1-2$ mM (ref. 17 and unpublished data). Indeed, growing cells contain UDPGlcNAc concentrations (Fig. 2) over 200-fold[†] greater than the inhibitable amidotransferase apparent K_i but at, or slightly above, the chitin synthetase apparent K_m . During hard times (sporulation and zoospore phases of the life cycle), net chitin accumulation is curtailed (Fig. 1) and, thus, a good-times mode of regulating UDPGlcNAc level (i.e., withdrawal into chitin) is effectively removed; in other words, once net chitin synthesis is terminated (or at least severely depressed), the organism is faced with developing some other way(s) to prevent itself from endlessly accumulating UDPGlcNAc.

Perhaps, then, the adaptive significance of endproduct inhibibility in the present instance is to help avoid the above metabolic predicament. During those phases of the life cycle when utilization of endproduct for chitin synthesis is either very low (sporulation) or nonexistent (zoospores), the effect of endproduct regulation is obviously to increase greatly the rate limitation of amidotransferase activity with respect to the three other hexosamine pathway enzyme activities (Fig. 3). Conversely, endproduct regulation is discarded and thereby the rate limitation is greatly decreased when chitin synthesis is required (growth phase). This intracellular coordination between hexosamine biosynthesis and chitin biosynthesis is all the more impressive in view of evidence that *throughout* the life cycle the four hexosamine pathway enzyme activities are located differently in the cell than is chitin synthetase activity (ref. 4 and unpublished data).

The data have also revealed a discoordination between hexosamine and chitin biosyntheses during the transition from the growth phase to the sporulation phase. Net chitin synthesis is quickly curtailed but the changeover from noninhibitable (or very weakly inhibitable) to inhibitable amidotransferase is gradual and continuous (Fig. 4). As would thus be predicted, an "overshoot" in UDPGlcNAc level is observed during the transition (Fig. 2). We do not understand the mechanism(s) involved in readjusting this overshoot level back to the UDPGlcNAc level eventually observed in the progeny zoospores. However, there is an intriguing possibility; a major use of UDPGlcNAc *only* during sporulation may be for the construction of a sporulation phase-specific structure, the discharge papilla, through which progeny zoospores eventually exit. Sikkema and Lovett (18) have recently reported that a glycoprotein is synthesized during papilla formation which is probably a major constituent of the papillary structure. Even with this possibility, another unexplained phenomenon is revealed by our data. If the large net consumption of UDPGlcNAc during the latter phases of sporulation (Fig. 2) involves utilization for macromolecular (glycoprotein?) synthesis, it would be expected to result in corresponding elevations in uridine nucleoside phosphate levels; this is not observed (Fig. 2).

Two points of broader interest derive from the above considerations. The first has to do with regulation of the hexosamine pathway in various organisms. Eukaryotes including microbial (19), plant (20), and animal sources (21-25), have exhibited endproduct-inhibitable amidotransferase activity, though variable in kinetic form; prokaryotes (25) have failed to do so. The obvious suggestion is that endproduct-inhibitable amidotransferase has not been observed in the prokaryotes because enzyme activity only from growing cultures (good times) has been analyzed.

The second point is as follows. In good-times situations, production of low molecular weight end products (e.g., amino acids, nucleotides, nucleotide-linked sugars, etc.) is metaboli-

cally coupled to utilization of such end products for macromolecular syntheses. Particularly when the apparent K_m values of the end products as substrates for macromolecular synthesis are significantly higher than their apparent K_i values as feedback inhibitors of their own synthesis, the good-times substrate requirements for macromolecular syntheses should prohibit the operation of endproduct inhibition. Conversely, during hard times, when the relevant macromolecular syntheses are severely depressed, effective means of controlling overproduction of end product should become a serious issue for the metabolic economy of the organism. It is from this more general framework that the present work suggests that regulation of biosynthetic pathways in other organisms might repay examination with respect to life cycle variation at the organismic level as well as to reproducibly changing developmental or physiological states within specific parts of multicellular organisms.

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