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Folic Acid and Pterin Deaminases in *Dictyostelium discoideum*: Kinetic Properties and Regulation by Folic Acid, Pterin, and Adenosine 3',5'-Phosphate

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Kinetic data obtained for deamination of pterin by the extracellular fraction from *Dictyostelium discoideum* yielded apparently linear Lineweaver-Burk plots for pterin. The Michaelis constant for pterin was 30 μM. The data for folic acid deamination yielded convex Lineweaver-Burk plots. Convex Lineweaver-Burk plots could result from the presence of two types of enzymes with different affinities. The data for folic acid deamination were analyzed mathematically for two types of enzymes. This analysis produced Michaelis constants for folic acid of 1.8 and 23 μM. Competition studies suggested that an enzyme with low affinity nonspecifically catalyzed the deamination of folic acid and pterin, whereas an enzyme with high affinity was a specific folic acid deaminase. A specific folic acid deaminase with high affinity appeared to be present on the surface of *D. discoideum* cells. The Michaelis constant for this enzyme was 2.6 μM. Cells growing in nutrient broth and cells starved in phosphate buffer released folic acid and pterin deaminases. The quantity of deaminase activities released by the cells appeared to be controlled by chemoattractants. Starving cells that were supplied with folic acid, pterin, or adenosine 3',5'-phosphate increased their extracellular folic acid and pterin deaminase activities to a larger extent than did cell suspensions to which no chemoattractants were added. Administration of folic acid or pterin to starving cells caused increases of the activity of extracellular adenosine 3',5'-phosphate phosphodiesterase and repressed increases of the activity of phosphodiesterase inhibitor.

Cells of *Dictyostelium discoideum* respond chemotactically to cyclic AMP (4, 16) as well as to folic acid (21, 22) and pterins (22). During cell differentiation from the growth phase to the aggregation-competent state, the chemotactic sensitivity of the cells to cyclic AMP increases (4), and that to folic acid and pterins decreases (22). The presence of receptors for cyclic AMP (10, 11, 18) and for folic acid (33) at the cell surface has been demonstrated. The folic acid receptors appear to be specific for folic acid and do not bind pterin (33).

Dictyostelium cells inactivate their chemoattractants in enzyme-catalyzed reactions. Cyclic AMP is hydrolyzed to 5'-AMP by means of cyclic AMP phosphodiesterases (for references, see reference 8), and folic acid and pterin most likely are hydrolytically deaminated to the corresponding lumazine forms by means of folic acid and pterin deaminases (23). Cyclic AMP phosphodiesterases as well as folic acid and pterin deaminases occur as extracellular and membrane-bound forms (8, 23). The activity of extracellular cyclic AMP phosphodiesterase is controlled by a protein inhibitor (26) which is also released by the cells.

Cyclic AMP and folic acid, when supplied as repeated pulses, stimulate cell differentiation from the growth phase to the aggregation-competent state (6, 7, 9, 34-36). A continuous supply or single additions of cyclic AMP or folic acid have little or no stimulatory effect (6, 9, 34, 36).

Externally added cyclic AMP also causes strong increases in the extracellular phosphodiesterase activity (12, 14, 15, 30, 36) and represses the increase in activity of phosphodiesterase inhibitor (15, 30, 36). Not only repeated pulses but also single additions as well as a continuous supply of cyclic AMP regulate the phosphodiesterase inhibitor system (14, 30, 36). The regulation by cyclic AMP of the phosphodiesterase and its inhibitor appears to be independent of the regulation of cell differentiation to aggregation competence (12, 36).

During preparation of this manuscript, Kakebeeke et al. (13) reported some kinetic properties of folic acid deaminases from *D. discoideum*. Bernstein and van Driel (2) pointed out that

externally added cyclic AMP pulses caused accumulation of increased amounts of extracellular folic acid deaminase activity. Rossier et al. (27) described regulation of the cyclic AMP phosphodiesterase inhibitor system by added folic acid. We report our results here because they extend those published previously (2, 13, 27). We investigated the kinetics of deamination of pterin in addition to that of folic acid and determined the specificities of extracellular and cell surface-bound deaminases for pterin and folic acid. We studied the effect of added folic acid and pterin, in addition to that of cyclic AMP, on the accumulation of extracellular folic acid and pterin deaminase activities. We show that accumulation of extracellular deaminase activities depends on de novo protein synthesis. Experiments performed with the developmental mutant agip 53 suggest that the regulation of accumulation of deaminase activities does not depend on differentiation to aggregation competence. We also examined the effect of pterin, in addition to that of folic acid, on the regulation of the cyclic AMP phosphodiesterase system.

MATERIALS AND METHODS

Chemicals. Folic acid (pteroylmonoglutamic acid), lumazine, and xanthine were obtained from Serva, Heidelberg, West Germany. Pterin was the product of Sigma Chemical Co., St. Louis, Mo. [3′,5′,7,9-^{3}H]folic acid (specific activity, 29 to 65 Ci/mmol) was purchased from Amersham Buchler, Braunschweig, West Germany. Pterin from Sigma was tritiated by Amersham (tritium labeling service). The product had a specific activity of 57 mCi/mmol. Guanine, cyclic AMP, 5′-AMP, cycloheximide, alkaline phosphatase (EC 3.1.3.1), and adenosine deaminase (EC 3.5.4.4) were obtained from Boehringer Mannheim GmbH, Mannheim, West Germany. 2-Deamino-2-hydroxyfolic acid was prepared as described (33). A sample of 2-deamino-2-hydroxyfolic acid was the generous gift of R. B. Angier, American Cyanamid Co. (Lederle Laboratories Division, Pearl River, N.Y.).

Culture conditions. *D. discoideum* strain Ax-2 as well as a morphogenetic mutant of strain Ax-2, agip 53 (6), were used. A clone of agip 53 was generously supplied by P. Brachet, Pasteur Institute, Paris. Cells were cultivated on nutrient medium supplemented with 1.8% maltose (31). Streptomycin (20 μg/ml) was added to cultures of agip 53. Cells were harvested at densities of 3×10^6 to 8×10^6 cells/ml, washed three times in the cold with 17 mM Sørensen phosphate buffer (pH 6.0), adjusted to 1×10^7 or 2×10^7 cells/ml, and shaken at 23°C. Time (in hours) of starvation in phosphate buffer is designated t_n.

At t_8, Ax-2 cells showed the characteristics of the aggregation-competent state: they formed tight EDTA-resistant aggregates (3) and, when placed on a microscope slide, streams of elongated cells oriented toward aggregation centers were observed. Agip 53 cells normally do not develop any of the characteristics of aggregation competence (6, 12).

Treatment of cells with chemoattractants. At t_0, cells (10^7/ml) were divided into 80-ml portions in 1-liter Erlenmeyer flasks and shaken until t_8 at 23°C. Unless stated otherwise, folic acid, pterin, or cyclic AMP was added to the cell suspensions either as pulses of 10^{-6} M (final concentration) at intervals of 6 min (small pulses) or as pulses of 10^{-4} M every hour (large pulses). Control cells were kept under identical conditions in the absence of added chemoattractants. Every hour, 1-ml samples were withdrawn from the cell suspensions and centrifuged, and the supernatants were assayed for activities of folic acid and pterin deaminase, cyclic AMP phosphodiesterase, and phosphodiesterase inhibitor.

Assays for folic acid and pterin deaminase activity. The activities of extracellular folic acid and pterin deaminases were assayed by UV spectrophotometry by a procedure analogous to that described for the assay of the deamination of pterin 6-carboxylic acid (17). The reaction mixtures (2 ml) contained 0.05 to 0.2 ml of *D. discoideum* supernatant and 17 mM Sørensen phosphate buffer, pH 6.0. Reactions were initiated by addition of folic acid or pterin (final concentrations varying between 2.5 and 100 μM). The temperature was 23°C.

For UV spectrophotometry at constant wavelength as well as for recording UV absorption spectra, a Zeiss DM4 spectrophotometer connected to a W+W recorder was used. Differences in the extinction coefficients between folic acid and 2-deamino-2-hydroxyfolic acid as well as between pterin and lumazine were determined for selected wavelengths at pH 6.0 and 23°C (Table 1). The rate of deamination of folic acid in the presence of pterin was determined at 287 nm, an isosbestic point for pterin and lumazine. The deamination of pterin in the presence of folic acid was assayed at 344.5 nm, an isosbestic point for folic acid and 2-deamino-2-hydroxyfolic acid. UV spectrophotometry was also used for the assay of folic acid and pterin deaminase activities in membranes, prepared according to Condeelis (5).

To determine whether folic acid was degraded by light applied in the spectrophotometric assay, folic acid solutions (5×10^{-6} and 5×10^{-5} M concentrations) were kept in the spectrophotometer at wavelengths of 280, 287, and 355 nm for 10 min. This treatment did not change their UV spectrum, indicating that folic acid was not degraded. Analysis of the incubation mixture of folic acid and *D. discoideum* supernatant

TABLE 1. *Differences in the extinction coefficients between folic acid and 2-deamino-2-hydroxyfolic acid and between pterin and lumazine at selected wavelengths*

Wavelength (nm)	Δε (cm²/mol × 10⁶)	
	Folic acid–2-deamino-2-hydroxyfolic acid	Pterin–lumazine
270	ND[a]	11.2
280	8.3	ND
287	5.8	0
344.5	0	2.2
355	3.2	4.4

[a] ND, Not determined in detail.

by thin-layer chromatography and UV absorption spectrophotometry indicated that the only product was 2-deamino-2-hydroxyfolic acid, as previously described (23).

The activity of cell surface-bound folic acid deaminase was assayed as described earlier (33) except that the cell density was 10^6 cells/ml and the temperature was 23°C. The technique involves deamination of [^{3}H]folic acid and separation of [^{3}H]folic acid from the reaction product [^{3}H]2-deamino-2-hydroxyfolic acid by paper chromatography (Whatman no. 1). The same technique was used for the assay of pterin deaminase activity using [^{3}H]pterin. The cell density was 10^7 cells/ml. [^{3}H]pterin and [^{3}H]lumazine were separated by paper chromatography with 0.5% Na_2CO_3 as the solvent. R_f values of pterin and lumazine were 0.57 and 0.69, respectively. This technique was also used for assaying extracellular pterin deaminase activity in the presence of folic acid.

Assay for activities of cyclic AMP phosphodiesterase and phosphodiesterase inhibitor. The activities of cyclic AMP phosphodiesterase and phosphodiesterase inhibitor were determined at 37°C in 70 mM Tris buffer, pH 7.4, according to published procedures (26). One inhibitor unit is defined as that quantity which in a total volume of 300 μl inhibits a phosphodiesterase activity of 2 nmol/min to one-half (26).

RESULTS

Kinetic properties of extracellular folic acid and pterin deaminases. Supernatants prepared from suspensions (2×10^7 cells/ml) at t_3 were used as a source of extracellular folic acid and pterin deaminases. Kinetic data obtained for pterin deamination yielded apparently linear Lineweaver-Burk plots. Table 2 gives values of maximal velocity and Michaelis constant obtained from six experiments.

Kinetic data obtained for folic acid deamination yielded curvilinear (convex) Lineweaver-Burk plots. This result was obtained in each of five experiments. Figure 1 gives means of the data of the five experiments. Convex Lineweaver-Burk plots can be interpreted in terms of negative cooperative interactions between catalytic sites or in terms of two or more types of catalytic sites with different affinities. We analyzed the data for two types of catalytic sites according to the equation:

$$v = v_1 + v_2 = \frac{V_1}{1 + \frac{s}{K_1}} + \frac{V_2}{1 + \frac{s}{K_2}}$$

Secondary data obtained in this analysis are also plotted in Fig. 1. Table 2 presents values for maximal velocities (V_1 and V_2) and Michaelis constants (K_1 and K_2).

Folic acid and pterin competed for the catalytic sites of the deaminases. Lineweaver-Burk analysis of the kinetic data for pterin deamination in the presence of 50 μM folic acid yielded typical competitive inhibition. Table 2 shows the value of the inhibition constant for folic acid obtained in four independent experiments.

Competition of pterin for deamination of folic acid was of a complex nature (Fig. 2). At higher folic acid concentrations, the Lineweaver-Burk diagram obtained for folic acid deamination in the presence of 50 μM pterin showed a larger slope than the diagram obtained in the absence of pterin. At lower folic acid concentrations, the slopes of both diagrams were similar. These results suggest competition of pterin for the low-affinity component of folic acid deaminase but no (or little) competition for the high-affinity component.

The folic acid deaminase reaction and the pterin deaminase reaction showed similar temperature dependence. Activities determined between 9 and 33°C yielded linear Arrhenius plots from which activation energies of 9 to 10 kcal (38 to 42 kJ)/mol were obtained.

Pterin and folic acid deaminases are different from guanine deaminase (EC 3.5.4.3). Supernatants obtained from t_3 suspensions did not cata-

TABLE 2. *Kinetic constants of extracellular and cell surface-bound folic acid and pterin deaminases*

Deaminase fraction	Substrate	Inhibitor	Maximal velocity (nmol/min per ml)	Michaelis constant (μM)	Inhibition constant (μM)
Extracellular[a]	Pterin		28 ± 12	30 ± 8	
	Folic acid		12.5	1.8	
			26	23	
	Pterin	Folic acid			32 ± 12
	Folic acid	Pterin			Complex inhibition
Cell surface bound[b]	Folic acid		0.44[c]	2.6	
	Folic acid	Pterin			No inhibition

[a] Prepared from suspensions of 2×10^7 cells/ml at t_3.

[b] Deamination of folic acid by the cell surface-bound enzyme was investigated at t_1 with cells freshly washed and suspended to 10^6 cells/ml.

[c] Expressed as nanomoles per minute per 10^{-6} cells.

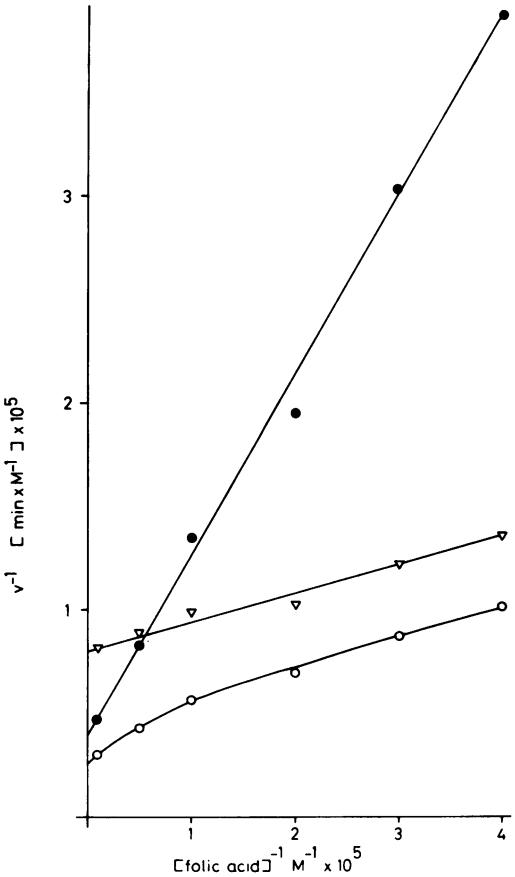

FIG. 1. *Lineweaver-Burk plot for deamination of folic acid by extracellular fraction. Data represent means of five experiments. From primary data* (○), *secondary data* (▽, ●) *were computed, assuming two types of catalytic sites with different affinities.*

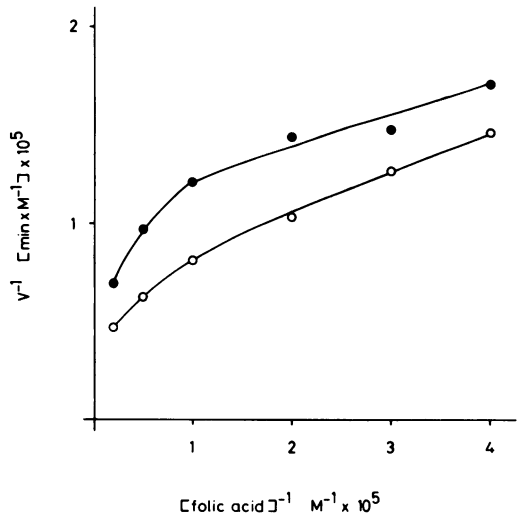

FIG. 2. *Competition of pterin for deamination of folic acid by extracellular fraction. Lineweaver-Burk plot for deamination of folic acid in the absence* (○) *and in the presence* (●) *of 50 μM pterin. Data represent means of two experiments.*

lyze the deamination of guanine to xanthine.

Kinetic properties of cell surface-bound folic acid deaminase. Kinetic properties of cell surface-bound folic acid deaminase were determined with washed cells at t_1. Data obtained for folic acid deamination yielded apparently linear Lineweaver-Burk plots (Fig. 3). Table 2 contains kinetic constants obtained from these data. The cell surface-bound enzyme appeared to be specific for folic acid. Pterin (100 μM) did not compete for folic acid deamination (Fig. 3).

The presence of pterin deaminase activity on the surface of living cells could not be reliably established. However, in membranes purified from t_1 cells, pterin deaminase activity of 4 nmol/min per mg of protein was measured. This activity was considerably smaller than that of folic acid deaminase, 50 nmol/min per mg of protein, measured in the same membrane preparations.

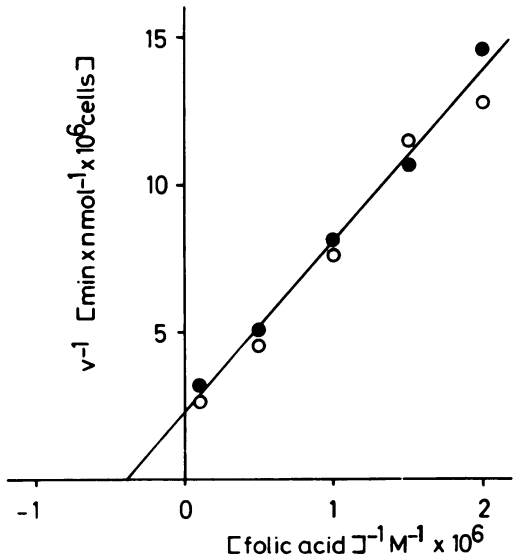

FIG. 3. *Kinetic properties of cell surface-bound folic acid deaminase. Lineweaver-Burk plot for deamination of folic acid in the absence* (○) *and in the presence* (●) *of 100 μM pterin.*

Activity of cell surface-bound folic acid deaminase during starvation. Cell surface-bound folic acid deaminase was present in undifferentiated cells just after separation from the growth medium. These results suggest that this enzyme is present on the surface of growing cells. The activity of cell surface-bound folic acid de-

aminase slightly decreased during starvation (Fig. 4).

Activities of extracellular folic acid and pterin deaminases during growth and starvation. Cells growing in nutrient medium released folic acid and pterin deaminases. In cultures of Ax-2 cells grown to densities of 1.4×10^6 and 6.3×10^6 cells/ml, folic acid deaminase activities of 9 and 25 nmol/min per ml, respectively, and pterin deaminase activities of 7 and 28 nmol/min per ml, respectively, were determined.

After separation from the growth medium, cells starving in phosphate buffer released folic acid and pterin deaminase activities (Fig. 5). Both activities increased with similar time courses. In the experiment shown in Fig. 5, the largest increase in enzyme activities occurred between t_1 and $t_{1.75}$. Between t_4 and t_8, further increases in deaminase activities were small. At $t_{3.5}$, part of the cells were washed and resuspended. The increases in deaminase activities in supernatants of washed cells between t_4 and t_8 were small (Fig. 5), comparable to those in supernatants of unwashed cells.

Regulation of extracellular folic acid and pterin deaminases by folic acid, pterin, and cyclic AMP. Cell suspensions that were supplied with folic acid increased their extracellular folic acid and pterin deaminase activities to a larger extent than did cell suspensions that were kept in the absence of added folic acid (Fig. 6). Comparable effects were caused by pulses of 100 or 10 μM folic acid added every hour as well as by pulses of 1 or 0.1 μM supplied at intervals of 6 min.

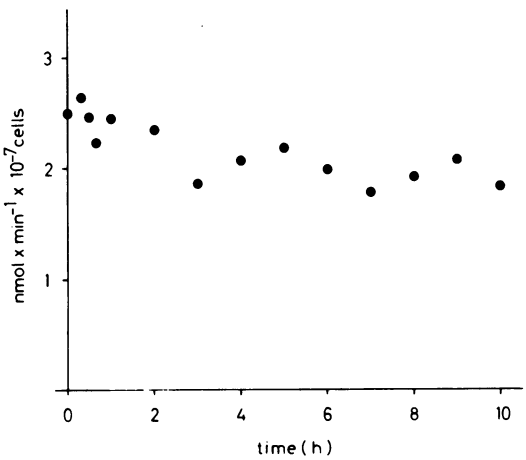

FIG. 4. *Activity of cell surface-bound folic acid deaminase during starvation. Activity was determined in the presence of 50 μM folic acid. Similar results were obtained in a second experiment.*

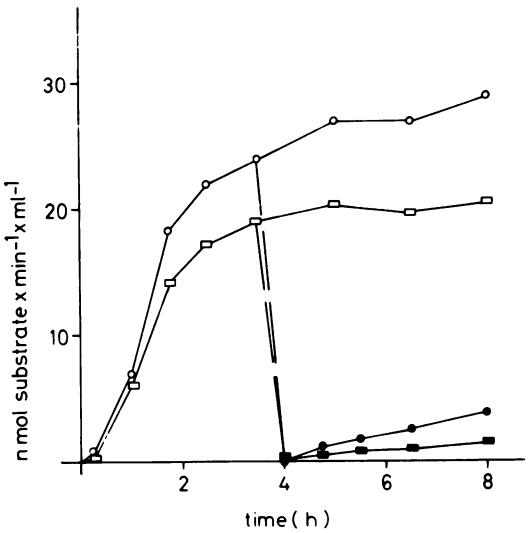

FIG. 5. *Accumulation of extracellular folic acid and pterin deaminase activities during starvation. The density of the cell suspension was 10^7 cells/ml. At $t_{3.5}$ part of the cell suspension was centrifuged, and the cells were washed and resuspended in the original volume of Sørensen phosphate buffer. Folic acid deaminase activity (○, ●) and pterin deaminase activity (□, ■) were determined in the presence of 100 μM folic acid and 100 μM pterin, respectively. The experiments were repeated twice with similar results.*

The increase in the activities of folic acid and pterin deaminases, both in the absence and in the presence of externally added folic acid, were reversibly blocked by cycloheximide (Fig. 6). These results suggest that the increases in deaminase activities, both in the absence and in the presence of added folic acid, depend on de novo protein synthesis.

Externally added pterin and cyclic AMP, like folic acid, caused accumulation of folic acid and pterin deaminase activities above the level of control suspensions to which no chemoattractants were added. Experiments were performed with strain Ax-2 and with the developmental mutant agip 53. We extended our study to agip 53 cells to examine whether the regulation of accumulation of deaminase activities depends on differentiation to aggregation competence. Agip 53 cells do not differentiate to the aggregation-competent state unless they are treated with small pulses of cyclic AMP (6, 12). Small pulses of folic acid and pterin as well as large pulses of cyclic AMP, folic acid, and pterin had little (if any) stimulatory effect on differentiation to aggregation competence. The time courses of accumulation of deaminase activities were similar in agip 53 cells that differentiated and cells that did not differentiate to aggregation compe-

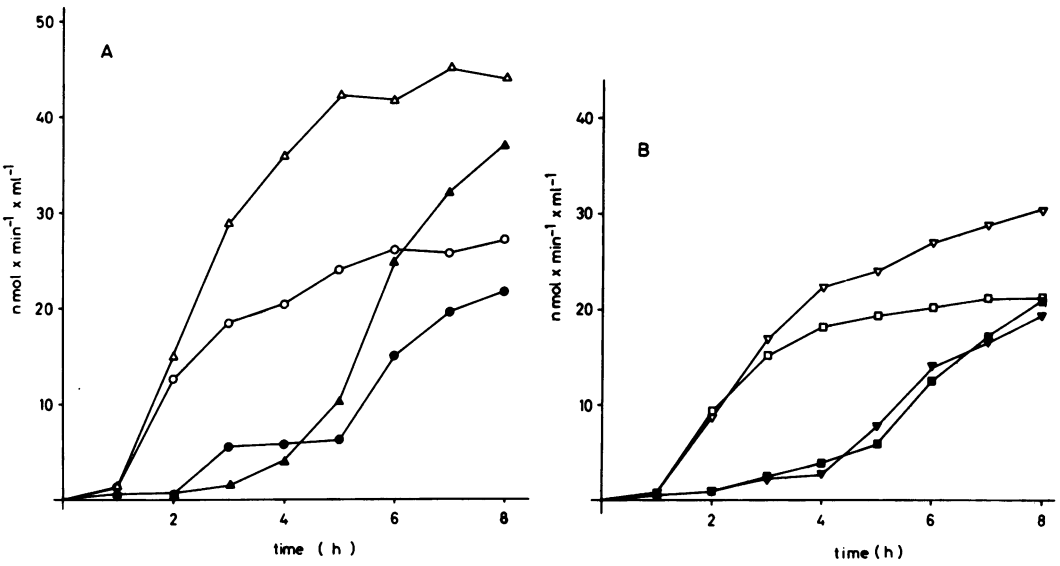

FIG. 6. *Accumulation of extracellular deaminase activities in the absence and in the presence of added folic acid and inhibition of the accumulation by cycloheximide. (A) Folic acid deaminase activity; (B) pterin deaminase activity. At t_0, a cell suspension (10^7 cells/ml) was divided into four equal parts. Two parts were kept in the absence of added folic acid (○, ●; □, ■) the other two parts were supplied with large pulses of folic acid (△, ▲; ▽, ▼). One part of each group was kept in the absence of cycloheximide (open symbols). At t_0, 250 μg of cycloheximide per ml was added to the other part (filled symbols). At t_4, cycloheximide was removed by washing and resuspending the cells in the original volume of Sørensen phosphate buffer. Similar results were obtained in a second experiment.*

tence (Fig. 7A and B). After an initial delay, steep increases in deaminase activities were observed. Between t_5 and t_8, further increases in deaminase activities were small. Large pulses and small pulses of all three chemoattractants caused comparable accumulation of deaminase activities.

Control experiments showed that the chemoattractants folic acid, pterin, and cyclic AMP did not cause elevated deaminase activities when added to cell-free supernatants instead of cell suspensions. When added to cell suspensions, the chemotactically inactive reaction products of the three chemoattractants, namely, 2-deamino-2-hydroxyfolic acid, lumazine, and 5'-AMP, did not stimulate the increase in deaminase activities.

To be able to compare quantitatively the results of different experiments, we calculated for each experiment mean values of deaminase activities between t_5 and t_8. Table 3 summarizes mean values of folic acid and pterin deaminase activities obtained with Ax-2 and with agip 53. Since all three chemoattractants caused comparable elevation of deaminase activities and since the effect of large pulses of chemoattractants was similar to that of small pulses, all experiments with chemoattractants were combined. In Ax-2, added chemoattractants caused elevated activities of folic acid deaminase and pterin deaminase of 88 ± 20 and 51 ± 21%, respectively. In all 10 experiments, elevation of activity was larger for folic acid deaminase than for pterin deaminase. In agip 53, added chemoattractants caused elevated activities of folic acid and pterin deaminases of 162 ± 78 and 204 ± 87%, respectively. Elevation of activities of folic acid and pterin deaminases was larger in agip 53 than in Ax-2.

Regulation of extracellular cyclic AMP phosphodiesterase and phosphodiesterase inhibitor by folic acid and pterin. We also examined whether externally added folic acid and pterin, like cyclic AMP (12, 14, 15, 30, 36), regulated accumulation of cyclic AMP phosphodiesterase and phosphodiesterase inhibitor. Small pulses of folic acid or pterin to agip 53 suspensions caused increases in the activity of cyclic AMP phosphodiesterase and repressed increases in the activity of phosphodiesterase inhibitor (Fig. 7C,D). The time course of accumulation of phosphodiesterase activity was similar in the presence of each of the chemoattractants folic acid, pterin, and cyclic AMP.

DISCUSSION

Kinetic data obtained for deamination of pterin by the extracellular fraction from *D. dis-*

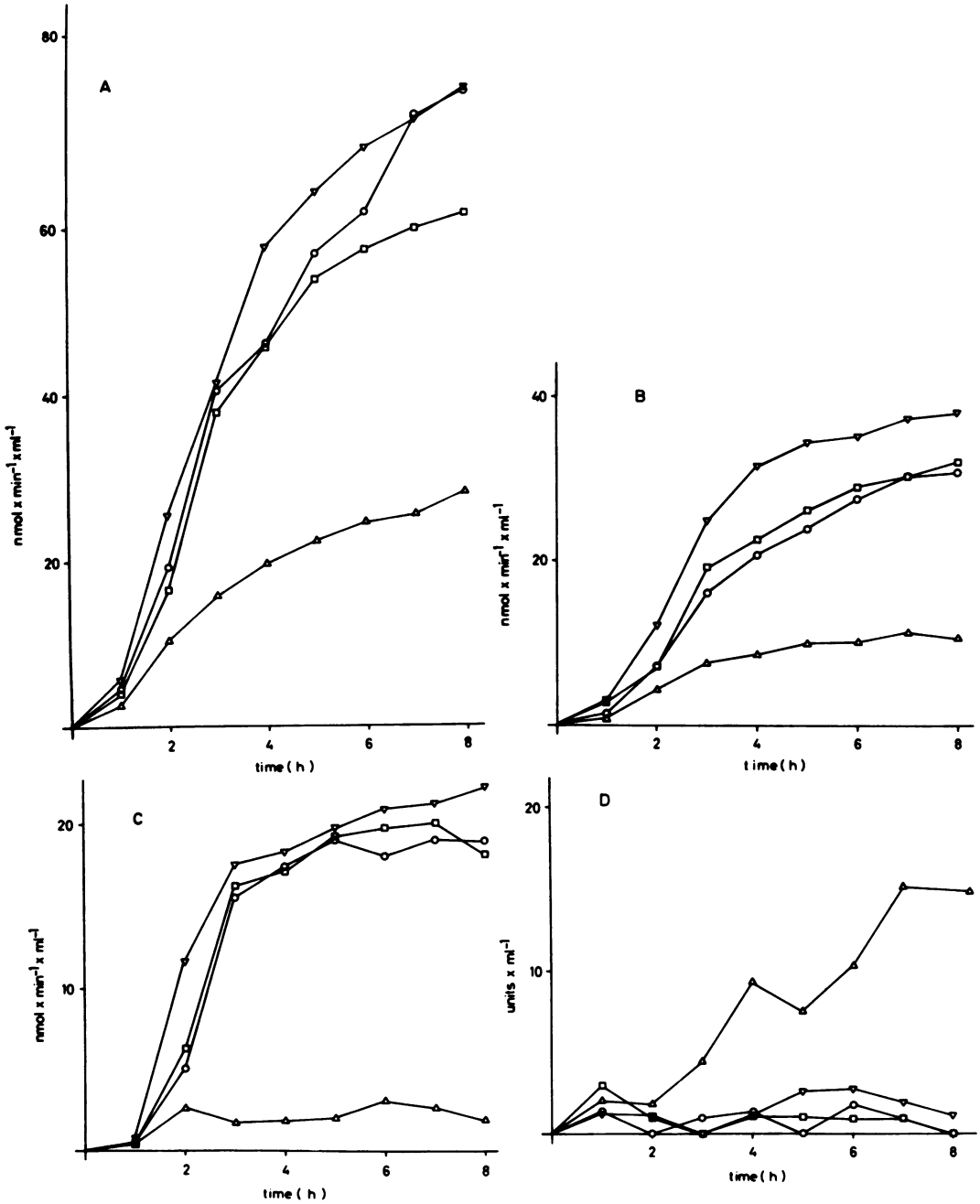

FIG. 7. *Regulation of folic acid and pterin deaminases, cyclic AMP phosphodiesterase, and phosphodiesterase inhibitor by applied folic acid, pterin, and cyclic AMP. A suspension of agip 53 cells (10^7 cells/ml) was divided into four equal parts. One part was kept in the absence of added chemoattractants (△). The second part received small pulses of folic acid (○), the third part received small pulses of pterin (□), and the fourth part received small pulses of cyclic AMP (▽). (A) Folic acid deaminase activity; (B) pterin deaminase activity; (C) cyclic AMP phosphodiesterase activity; (D) phosphodiesterase inhibitor activity.*

coideum yielded apparently linear Lineweaver-Burk plots for pterin. The data for folic acid deamination yielded convex Lineweaver-Burk plots. Convex Lineweaver-Burk plots may result from the presence of two types of enzymes with different affinities. The data for folic acid deam-

TABLE 3. *Regulation of extracellular folic acid and pterin deaminases by added chemoattractants*[a]

Cell type	Added chemoattractant	Folic acid deaminase		Pterin deaminase		No. of expt
		Activity (nmol/min per ml)	Elevation of activity (%)	Activity (nmol/min per ml)	Elevation of activity (%)	
Ax-2	None	17.5 ± 7.9		13.8 ± 6.0		6
Ax-2	Folic acid, pterin, or cyclic AMP	33.5 ± 16.3	88 ± 20	19.7 ± 6.7	51 ± 21	10
Agip 53	None	24.9 ± 4.5		10.5 ± 3		5
Agip 53	Folic acid, pterin, or cyclic AMP	65.7 ± 17.4	162 ± 78	31.6 ± 6.3	204 ± 87	12

[a] Data represent mean values ± standard deviation.

ination were analyzed mathematically for two types of enzymes. Support for the assumption of two types of enzymes came from competition studies. Folic acid competed for deamination of pterin, indicating a nonspecific deaminase for pterin and folic acid. The value of the inhibition constant for folic acid was similar to that of the Michaelis constant for folic acid of the proposed deaminase with lower affinity (Table 2). The pattern of the competition of pterin for deamination of folic acid (Fig. 2) suggested the presence of a specific folic acid deaminase with high affinity in addition to the nonspecific enzyme with lower affinity. A specific folic acid deaminase with high affinity appeared to be present on the cell surface of *D. discoideum*.

Recently Bernstein and van Driel (2) and Kakebeeke et al. (13) investigated the deamination of folic acid by the extracellular fraction from *D. discoideum*. Bernstein and van Driel (2) obtained a linear Lineweaver-Burk plot. These results contrast those described here and those reported by Kakebeeke et al. (13), who showed a nonlinear Eadie-Hofstee plot for folic acid deamination. The Michaelis constants given by Kakebeeke et al. (13) for low-affinity and high-affinity folic acid deaminases are smaller than the constants reported here. Differences might be due to different techniques and different strains used. Kakebeeke et al. (13) obtained a surprisingly small temperature dependence between 4 and 20°C of the activity of extracellular folic acid deaminases. This temperature dependence contrasts with that reported here.

Pterin deaminases have been described as occurring in a species of another genus of cellular slime molds, *Polysphondylium violaceum* (23), as well as in *Alcaligenes* species (17, 20), *Clostridium acidi-urici* (32), *Pseudomonas* species (24, 29), honeybee larvae (25), and rat liver (25). These enzymes catalyze the deamination of pterins (17, 20, 23–25, 29, 32) and folic acid (17, 20, 23, 24). So far a specific folic acid deaminase, comparable with the cell surface-bound *D. discoideum* enzyme, has not been described.

Cells growing in nutrient broth and cells starving in phosphate buffer released folic acid and pterin deaminases. The accumulation of deaminase activities in starving cell suspensions showed a sigmoidal time dependence. The time course reported here agrees with that shown by Bernstein and van Driel (2) but differs from that described by Kakebeeke et al. (13), who found the largest increase in extracellular deaminase activity during the first hour of starvation. The reactions that regulate the time-dependent accumulation of folic acid and pterin deaminases are not known. The accumulation of deaminase activities seems to involve de novo protein synthesis (Fig. 6). The amount of enzyme activities released per time interval decreased at later starvation times. This decrease apparently does not depend on the amount of activity present in the extracellular space (Fig. 5). Results obtained with the developmental mutant agip 53 indicate that the time course of accumulation of deaminase activities does not depend on differentiation to aggregation competence. Regulation of accumulation of deaminases may depend on differentiation steps of the preaggregation phase.

The quantity of deaminase activities released by the cells appears to be controlled by chemoattractants. Added chemoattractants caused elevated activities of extracellular folic acid and pterin deaminases. It is known that starving *D. discoideum* cells release cyclic AMP (1, 19) as well as small amounts of folic acid-related compounds (22). We cannot rule out the possibility that the accumulation of deaminase activities in the extracellular space observed in the absence of added chemoattractants is caused by the chemoattractants which the cells release. The accumulation of extracellular deaminase activities during growth could be caused by chemoattractants present in the nutrient yeast extract (21).

In *D. discoideum*, cyclic AMP serves as an acrasin which brings the cells together into multicellular masses (4, 16). So far there is no evidence that folic acid, pterin, or one of their derivatives acts as an acrasin. It has been suggested that attraction to folic acid and pterins may be a food-seeking device of the cells which naturally prey on folic acid- and pterin-releasing bacteria in the soil (22). The biological function

of folic acid and pterin deaminases as well as cyclic AMP phosphodiesterase apparently is reduction of the concentration of chemoattractants. Low background concentrations of chemoattractants would enable the cells to sense small concentration gradients (28). Compatible with such a biological function is the cells' ability to respond to high concentrations of folic acid and pterin with high activities of folic acid and pterin deaminases and to high concentrations of cyclic AMP with high activity of cyclic AMP phosphodiesterase and low activity of phosphodiesterase inhibitor. Surprising are the results that cyclic AMP, like folic acid and pterin, causes accumulation of increased amounts of deaminase activities and that folic acid and pterin, like cyclic AMP, regulate the cyclic AMP phosphodiesterase inhibitor system. One possible interpretation of these results is that all three chemoattractants activate the same intracellular pathway. One or more products of this pathway may induce folic acid and pterin deaminases as well as cyclic AMP phosphodiesterase and repress phosphodiesterase inhibitor.

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