THE REGULATION OF TYROSINE AMINOTRANSFERASE IN TETRAHYMENA PYRIFORMIS

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Summary. The activity of tyrosine aminotransferase in the ciliated protozoa Tetrahymena pyriformis is several-fold higher than in rat liver during all growth-phases of the organism grown in proteose-peptone or synthetic media. Glucose represses enzyme activity in all growth-phases while the absence of acetate greatly stimulates enzyme activity in log phase. In the presence of acetate enzyme activity increases 3-fold after the completion of log phase. Our data reveal the operation of regulatory mechanisms for tyrosine aminotransferase in T. pyriformis.

The activity of rat liver tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) is greatly stimulated by glucocorticoids (1) and glucagon (2). Indeed, stimulation of gluconeogenic activity in general by means such as high protein diets, starvation and alloxan diabetes results in high enzyme activity (3). The ciliated protozoa Tetrahymena pyriformis resemble liver in their enormous gluconeogenic capacity (4) and their nutritional requirements resemble those of the rat (5). Nutritional experiments have indicated the occurrence of transaminations (5) but to our knowledge no direct enzymological assay has been made in extracts of this organism. We now wish to report both the presence and the regulation of this enzyme in T. pyriformis.

Materials and methods. T. pyriformis, strain W, were obtained from the Culture Collection of Algae and Protozoa, Cambridge, England. The organisms were grown aerobically at $24^{\circ} \pm 1C$ in axenic culture without shaking in the following three media:

a) proteose-peptone (Difco) 2%, fortified with 0.1% yeast extract (Difco), b) the synthetic medium of Dewey et al. (6) but without glucose and Tween 85. The medium contains 16 amino acids (excluding tyrosine, cysteine, cystine, glutamine and hydroxyproline), vitamins, nucleotides, salts and acetate and will be referred to as the "basal medium" and c) the basal medium but without acetate. Glucose, when present, was autoclaved separately and added at a concentration of 0.25%. Stock cultures were maintained in 16 x 125 mm screw-cap culture tubes in 5 ml of proteose-peptone medium and transfers were made every 3-4 days. Cells were grown in 100 ml of medium in 300 ml nepheloflasks (Bellco) with Morton closures and a side-arm to measure optical density in a Coleman Junior II spectrophotometer. Aliquots were withdrawn aseptically and washed twice with cold distilled water. Cell breakage and extraction was accomplished most reproducibly by freezing the cell pellet at -45°C for 10 minutes and thawing after addition of 0.5 ml of 0.1M sodium phosphate buffer, pH 7.5. The disrupted cells were centrifuged for 15 minutes at 27,000 x g and tyrosine aminotransferase was assayed in 0.1 ml of the clear supernatant by the spectrophotometric procedure of Lin et al. (7). Protein was determined by the method of Lowry et al. (8).

Results and Discussion. Preliminary experiments with the proteose-peptone medium revealed the presence of enormous amounts of tyrosine aminotransferase in T. pyriformis. In the experiment shown in Table I, column 2, the specific activity of the enzyme in a growing culture at 136 hours was twice as high as that measured at 40 hours. The enzyme activity in normal rat liver was found to average 0.6 μmoles x 10^2/min/mg protein. Hence, the specific activity of the enzyme was 5 to 10-fold higher in T. pyriformis than in rat liver. Moreover, the activity was considerably re-

Hours of growth	Medium	
	Proteose-peptone	Proteose-peptone + glucose
40	3.35	1.49
50	2.98	1.09
64	2.73	1.11
74	3.30	1.46
88	3.41	1.18
112	5.37	2.24
136	6.88	4.76

Table I. The activity of tyrosine aminotransferase (μmoles x 10^2/min/mg protein) in a growing culture of T. pyriformis.

pressed in the presence of glucose (column 3). The use of the proteose-peptone medium was discontinued and in all subsequent experiments the synthetic media, in which log phase extends to about 45 hours, were used instead. The enzyme activity in the organisms grown in the synthetic media was lower than when they were grown in the proteose-peptone medium. A number of preliminary experiments confirmed that in the basal medium the activity of tyrosine aminotransferase rose steeply after the log phase had ended and the culture entered a slower growth rate. It was also consistently found that the enzyme activity in the absence of acetate was always higher after 30-50 hours of growth than in its presence.

To investigate the entire course, media were inoculated with cells from a culture growing in the basal medium in the presence of glucose with known low enzyme specific activity (0.37 μmoles x 10^2/min/mg protein). The growth curve and the time-course of specific activity in the basal medium are illustrated in fig. 1. At 24 hours the specific activity had risen three-fold and by 31 hours had subsided to a level that persisted for about 15 hours.

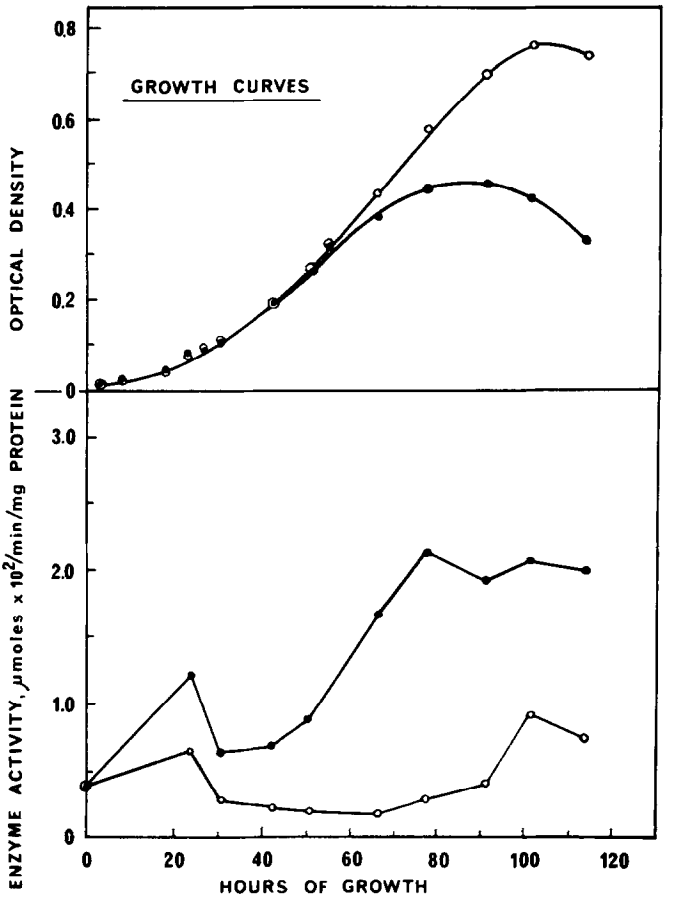

Fig. 1. The activity of tyrosine aminotransferase in T. pyriformis in all growth phases of the organism. Basal medium (-•——•-), basal medium plus glucose (-o——o-).

At the end of this period, growth ceased to be strictly logarithmic and the specific activity rose steeply to reach by about 80 hours a value three times as high as that prevailing from 30-45 hours. Thereafter, the activity remained constant during the relatively short stationary phase and the death phase. A modest early burst of activity occurred also in the presence of glucose but the subsequent course was entirely different and the activity fell to levels lower than that of the inoculum itself (fig. 1). A modest elevation occurred when the culture was nearing stationary phase, most probably due to diminishing glucose concen-

tration, followed by a last drop at 114 hours.

In the basal medium lacking acetate the activity in 24 hours rose to eight times the activity of the inoculum and it never really fell to low levels during log phase (fig. 2). Rather, it stabilized after what appear to be damped oscillations, at the level attained much later in the basal medium (fig. 1). The time course in the presence of glucose (fig. 2) was qualitatively the same as that obtained with the basal medium supplemented with glucose.

The experiment illustrated in figs. 1 and 2 reproduced all the

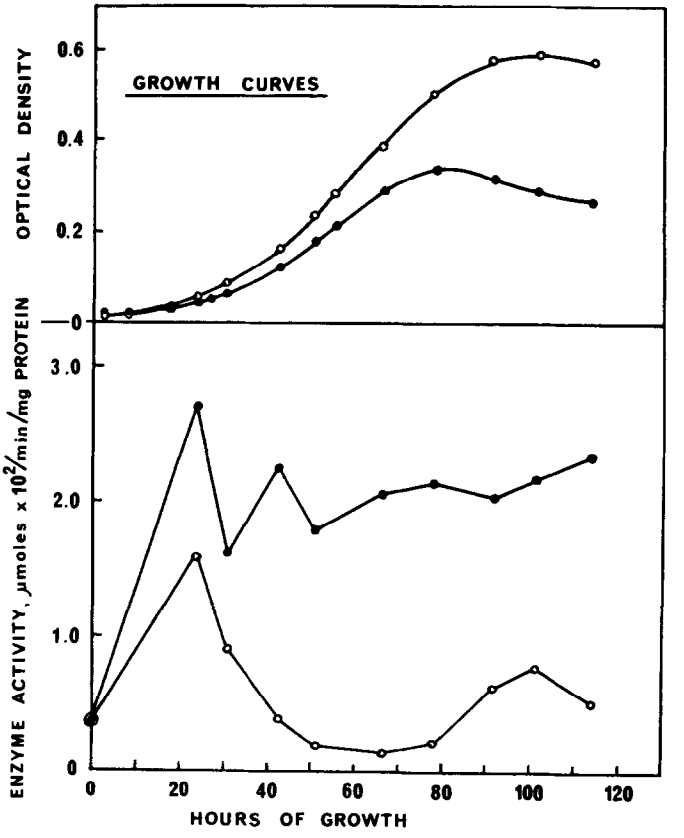

Fig. 2. The activity of tyrosine aminotransferase in T. pyriformis in all growth phases of the organism. Basal medium lacking acetate (-•——•-), basal medium lacking acetate and supplemented with glucose (-o——o-).

evidence collected in the initial exploratory experiments which can be summarized as follows: a) glucose represses enzyme activity in all media and all growth-phases, b) in the basal medium the enzyme activity rises steeply just after the completion of logarithmic growth to reach and remain at a level three times as high as the stabilized level of log phase, c) in the absence of acetate this high level is reached and even temporarily exceeded in log phase.

Our experiments demonstrate the operation in T. pyriformis of regulatory mechanisms which are dependent upon cellular metabolic activities. The latter are in turn determined by the growth-phase and the nutritional environment. The regulation of the enzymes of the glyoxylate cycle has been demonstrated (9). Thus acetate stimulates the enzymes of the cycle isocitrate lyase and malate synthase. Malate formed from acetate (as acetyl CoA) and glyoxylate can then enter the Embden-Meyerhof pathway and acetate is a major gluconeogenic precursor (10).

It is interesting to speculate on the metabolic activity responsible for the adaptive responses of tyrosine aminotransferase in T. pyriformis. As in the rat, they appear to be related with gluconeogenic activity (3). The increase of enzyme activity in the basal medium (fig. 1) occurs immediately after log phase when the rate of glycogen accumulation from non-sugar precursors (gluconeogenesis) steeply increases (11). In the absence of the gluconeogenic precursor acetate (fig. 2) the gluconeogenic potential of the organisms would have to be satisfied by an increased conversion of amino acids from the medium to Krebs-cycle intermediates. Thus, the higher levels of tyrosine aminotransferase during log phase in the absence of acetate coincide with an intensified flow of Krebs-cycle intermediates not shared by the glyoxylate cycle.

T. pyriformis does not utilize glucose for energy metabolism under aerobic conditions (12). We have also observed that the logarithmic growth rate (generation time) is not affected by the presence of glucose, only the final growth is increased. It is thus difficult to visualize the repressive effect of glucose (figs. 1, 2) as "catabolite repression" as defined by Magasanik (13). It is likely that, at least during very aerobic log phase, the repression is due to reduced gluconeogenesis in the presence of supplied glucose, rather than to a catabolite of glucose.

The above speculations may serve as working hypotheses in the study of the mechanisms involved at the molecular level.

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