AMPHI-DIRECTIONAL CONTROL OF A REVERSIBLE REACTION COMMON TO TWO ENZYME SEQUENCES

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Ornithine-δ-transaminase (OTA) catalyzes the only reversible reaction common to the metabolic pathways interconverting proline, glutamate and arginine. Studies on this enzyme in vivo suggest the existence of different mechanisms for control of its activity in liver and kidney and also indicate similarities in its regulation in kidney and in Chang's liver cells (Sawamura and Strecker, 1967; Volpe, Sawamura and Strecker, 1968). Because of the key position of ornithine-δ-transaminase in different interconnecting enzymatic pathways it was of interest to determine whether its control was "polarized" in response to the products at opposite ends of the metabolic sequences, namely arginine, proline and glutamate. Such a model might have relevance to studies of differentiation.

Methods. 21-day old rats were fed on defined diets for two weeks. The diets are listed under the appropriate table or figure. After two weeks the rats were sacrificed, livers and kidneys removed, homogenized in neutralized 0.25 M sucrose solution, frozen overnight, thawed and sonicated for 1 minute. Chang's liver cells were grown and harvested as described previously (Strecker and Eliasson, 1966). The harvested cells after washing were suspended in neutralized 0.25 M sucrose solution,

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frozen overnight, thawed and sonicated. Ornithine-δ-transaminase activity was determined as described previously (Strecker, 1965). Activity is expressed as μmoles of Δ^1-pyrroline-5-carboxylate (P5C) formed per hour ± standard deviation. Protein concentration was determined by the method of Lowry et al. (1951).

Results. On defined diets lacking arginine, ornithine-δ-transaminase activity in livers of weanling rats was increased as compared to that in livers of rats on the same diet containing arginine. Kimura et al. (1965) had reported a preliminary account of similar findings. A series of experiments with increasing concentrations of arginine in the diet demonstrated that the specific activity of ornithine-δ-transaminase decreased to a constant level at about 0.6% arginine.

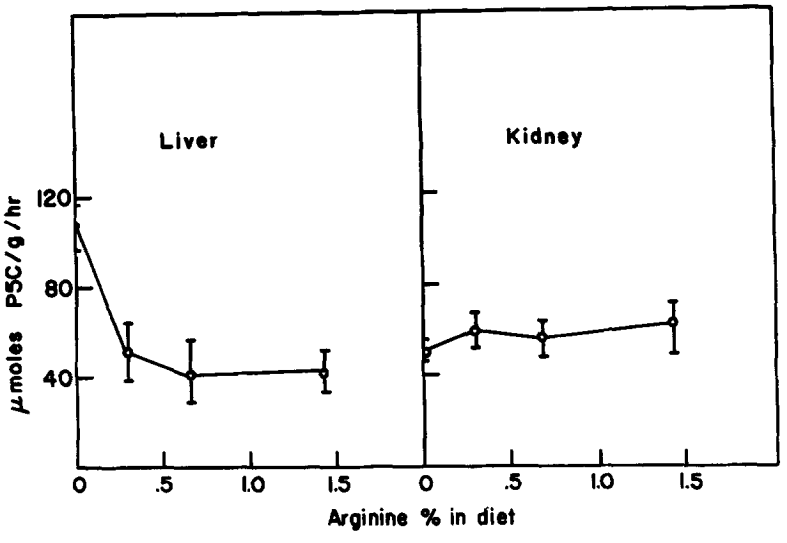

Fig. 1. Effect of dietary arginine on ornithine-δ-transaminase activity of liver and kidney of young rats. The diets contained 1.6% salt mix (U.S.P. XVII), 9% corn oil, 0.5% vitamin mix, 2% agar, 62% dextrin and the following amino acids in %: L-his, 0.45; DL-ile, 1.92; L-leu, 1.24; L-lys HCl, 2.5; DL-met, 1.32; DL-phe, 1.73; DL-thr, 1.82; DL-trp, 0.29; DL-val, 1.96; L-asp, 2.5 and DL-ala from 0.96 to 6.66 to maintain nitrogen equivalence as the original concentration of arginine was varied.

Higher concentrations of arginine did not have any further effect. Under the same conditions no change in activity of kidney OTA was obtained (Fig. 1).

Systematic experiments with the other amino acids indicated that arginine was the only one whose exclusion from the diet resulted in the increase of OTA activity seen in Fig. 1. However, these experiments also revealed that glycine in the diet further increased the activity of ornithine-δ-transaminase in livers of rats on diets which were low in arginine, but had no effect in diets in which the concentration of arginine was high enough to give maximal repression of activity (Table 1). As before, no effect was seen with kidney.

Table 1. Effect of glycine on ornithine-δ-transaminase activity of liver

% Arginine	μMoles P 5C/g/hr	
	- glycine	+ glycine
0	107 ± 11	159 ± 25
0.28	52 ± 14	72 ± 20
0.64	42 ± 15	35 ± 3

The diet was the same as for the experiments in fig. 1. The concentrations of glycine to maintain nitrogen equivalence were 2.82%, 2.33% and 1.79% respectively.

Another series of experiments in which amino acids were added in turn to a basal diet of 15% casein revealed that the specific activity of kidney OTA could be increased by adding groups of certain amino acids but excluding glutamate. The addition of glutamate to these groups resulted in repression of this increase of activity.

The effect of glutamate appeared to depend on the magnitude of the increase of activity. When added to diets which brought about comparatively small increases of ornithine-δ-transaminase activity, repression by glutamate was correspondingly less. Other amino acids including proline showed no repressing effect (Table II).

Table II. Repression by glutamate of OTA increase in kidney

Diet	μMoles P5C/mg prot/hr
15% Casein	0.39 ± 0.04
" + Asp, Gly, Ala	0.95 ± 0.05
" + Asp, Gly, Ala, Glu	0.55 ± 0.06
" + Leu, Ile, Val	0.86 ± 0.15
" + Leu, Ile, Val, Glu	0.69 ± 0.13

The diets contained in addition to salt mix, corn oil, vitamins and agar as in the experiments in fig. 1, 55% dextrin, 15% casein and the amino acids as follows: L-asp, 2.31; gly, 2.63; DL-ala, 3.11; L-glu (Na), 6.52; L-leu, 4.50; DL-ile, 5.78 and DL-val, 6.67.

Under the same conditions, these groups of amino acids did not increase the specific activity of liver OTA and there was no repression by glutamate.

Some factors regulating the level of OTA in Chang's liver cells in culture have been previously reported. These cells are unable to synthesize arginine but do convert arginine to proline and to glutamate. Arginine has no repressing effect on ornithine-δ-transaminase and the omission from the growth media of this amino acid as well as any other essential amino acids results in a decrease of ornithine-δ-transaminase. Glutamate and proline are not normal components of the culture media and we have previously reported that the addition of either of these amino acids to the growth media at 6mM concentration did not have a significant effect on ornithine-δ-transaminase (Strecker and Eliasson, 1966). We now find that higher concentrations of proline indeed decrease the activity per mg protein compared to the control experiment, although there is no change in the activity per cell (Fig. 2). This occurs because of an increased protein content of the cell without a corresponding increase of ornithine-δ-transaminase activity. Other non-essential amino acids such as serine, glycine, alanine and aspartate also increased cellular protein but enzyme activity increased correspondingly.

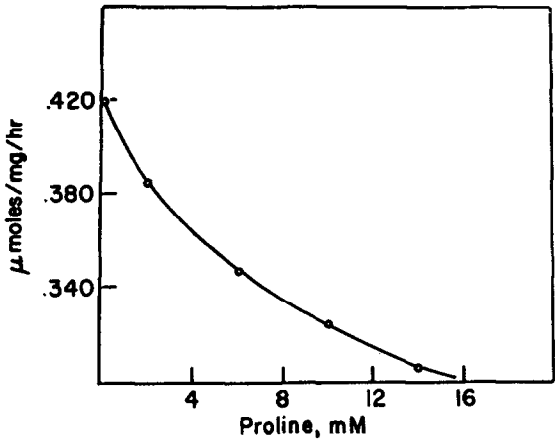

Fig. 2. Effect of proline on ornithine-δ-transaminase activity of Chang's liver cells harvested after 48 hours of growth.

Discussion. The finding that arginine but not glutamate or proline decreases ornithine-δ-transaminase in liver suggests that the direction of metabolic flux of the sequence interrelating glutamate, proline and arginine is chiefly in the direction of arginine synthesis in this organ. The finding that glutamate but not arginine decreases ornithine-δ-transaminase in kidney suggests that the metabolic sequence in this organ is chiefly in the direction of arginine utilization. These results indicate further that a reversible reaction may be amphi-directionally controlled and raise the question of whether the ornithine-δ-transaminase activities in the two tissues are catalyzed by the same protein and what sort of modifications of gene expression for this enzyme have taken place during the processes of differentiation to liver and kidney tissue. The experimental data also appear to demonstrate that the decreases of ornithine-δ-transaminase activity brought about by either arginine or glutamate, respectively, do not go below a minimum base level. This observation raises the question of whether, as is true for many enzymes in microorganisms, both repressible and non-repressible ornithine-δ-transaminase activities exist in each of these two organs. The data with the Chang's liver cells, a polyploid line derived from human liver, which shows similarities to kidney in the regulation of

ornithine-δ-transaminase, would suggest that these cells could provide a model for some aspects of gene modification leading to differentiation.

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