Effect of anaerobiosis and addition of keto acids on glutamine utilization by Ehrlich ascites-tumor cells

Ehrlich ascites-tumor cells metabolize glutamine rapidly with the formation of equivalent amounts of ammonia[1]. Anaerobiosis produced by partial vacuum or by nitrogen reduced glutamine utilization considerably. This effect has been studied and related to the effect of keto acids.

It was found that anaerobiosis did not irreversibly reduce glutamine utilization; glutamine disappearance and ammonia formation were markedly stimulated by air after pre-incubation for 1 h in N_2. It was also found that malonate (0.06 *M*) and 2,4-dinitrophenol (10^{-4} *M*) did not affect glutamine uptake appreciably. This suggested that glutamine uptake was not dependent upon intact oxidative phosphorylation. No significant L-glutamine oxidase activity could be detected in homogenates of ascites cells, and glutamine exerted no effect on O_2 uptake and CO_2 evolution by whole cells. The effect of anaerobiosis on glutamine uptake was also demonstrated in cell homogenates and in suspensions of mitochondria fortified with adenosine triphosphate. Thus the reduced anaerobic glutamine uptake was not explained by lower anaerobic permeability of the cell membrane for glutamine.

Glutamine which disappeared under anaerobic condition accumulated almost quantitatively as glutamate. Under aerobic conditions the glutamine taken up could be largely accounted for as glutamate and aspartate, but some alanine was also found. This suggested that the increased glutamine utilization under aerobic conditions was due to transaminase reactions.

Aspartate, alanine and glutamate were determined by quantitative paper chromatography, using a modification of REDFIELD's technique[2]. In addition, glutamine, glutamate and α-ketoglutarate were estimated enzymically[3,4].

In further experiments, it was found that oxaloacetate markedly stimulated the anaerobic glutamine utilization with the formation of equivalent amounts of α-Ketoglutarate and aspartate, plus some alanine (Table I). Pyruvate only slightly stimulated glutamine utilization, and α-ketoglutarate and alanine were found. α-ketoglutarate exerted no effect on glutamine utilization. Oxaloacetate also stimulated glutamine utilization by isolated mitochondria both under aerobic and anaerobic conditions. Oxaloacetate had little effect on glutamine utilization in the supernatant fraction of 20,000 × *g*, while pyruvate stimulated glutamine utilization almost to the same extent as in mitochondria. This suggested that two different transaminases were present, a mitochondrial bound transaminase involving oxaloacetate and a water-soluble transaminase involving pyruvate.

Thus, the results could either be explained by the combined action of glutamine transaminases and amidases or by the action of glutaminase I and glutamate transaminases. Glutaminase I as well as glutamate–aspartate transaminase and glutamate–alanine transaminase were also found in mitochondria in sufficient amounts to explain the effect of keto acids on glutamine utilization. However, oxaloacetate exerted no appreciable effect on the final glutamate concentration when glutamine was present; furthermore, the glutaminase I activity could be destroyed by heating cell homogenate for 10 min at 50°, but oxaloacetate still stimulated glutamine utilization. Therefore, it appears likely that ascites cells contained an active mitochondrial bound glutamine–aspartate transaminase and a less active water-soluble

TABLE I

THE EFFECT OF KETO ACIDS ON GLUTAMINE UTILIZATION

Washed ascites-tumor cells (0.8 ml packed cells), mitochondria (40 mg protein) and supernatant of 20,000 × *g* (50 mg protein) prepared from ascites-tumor cells, were incubated in air or in N_2. The N_2 had been washed in an alkaline pyrogallol solution. Additions (m*M*): L-glutamine, 8; potassium phosphate buffer pH 7.4, 10; tris(hydroxymethyl)aminomethane buffer pH 7.4, 10; where indicated, oxaloacetate, 25, pyruvate, 14, or *α*-ketoglutarate, 14 were added (sodium salts). Oxaloacetic acid was neutralized with Na_2CO_3 immediately before use. The final volume was 6 ml. The substrates were added after temperature equilibration for 10 min. The incubation was then continued for an additional 60 min. The whole cells were incubated at 37°, the cell fractions at 30°.

	Additions	*Gas phase*	*Glutamine disappearing (μmoles)*	*NH_3 (μmoles)*	*Glutamate (μmoles)*	*Aspartate (μmoles)*	*Alanine (μmoles)*	*α-keto-glutarate (μmoles)*
Whole cells	—	Air	31.4	36.0	14.8	10.9	8.4	0.2
	—	N_2	17.0	21.6	16.1	0.9	5.4	0.1
	Oxaloacetate	Air	38.5	41.3	15.8	14.5	11.5	5.0
	Oxaloacetate	N_2	48.0*	50.3	17.3	22.2	13.0	27.7
	Pyruvate	N_2	20.0	23.8	14.8	1.7	12.5	7.3
	α-ketoglutarate	N_2	16.2	20.3	17.5	1.2	6.0	—
Mitochondria	—	N_2	15.3	16.4	3.3	0	2.3	0
	Oxaloacetate	N_2	39.1	40.2	4.5	23.0	3.0	22.0
	Pyruvate	N_2	15.3	16.1	3.1	0	5.1	2.0
Supernatant	—	N_2	7.7	6.6	2.1	0	4.7	0
	Oxaloacetate	N_2	9.0	11.7	0.6	0	5.2	4.6
	Pyruvate	N_2	9.0	8.8	0.3	0	8.2	2.0

* Supply of glutamine exhausted.

glutamine–alanine transaminase. The latter enzyme might be identical with liver glutaminase II[5]. It has also been reported that oxaloacetate stimulates ammonia production from glutamine in liver extract, but the effect of oxaloacetate was somewhat lower than that of pyruvate, and both reactions occurred in the water-soluble fraction[6]. Thus this enzyme might be distinguished from that of ascites cells by its relative activity and solubility.

The results suggest that the reduced anaerobic glutamine utilization may be related to lack of oxaloacetate under anaerobic conditions.

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