# Channeling of ammonia from glutaminase to carbamoyl-phosphate synthetase in liver mitochondria

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In isolated rat-liver mitochondria the rate of citrulline synthesis from glutamine does not respond to changes in the ammonia concentration in the extramitochondrial fluid. This suggest that ammonia, produced in the mitochondria via glutaminase, is directly channeled to carbamoyl-phosphate synthetase.

*Carbamoyl-phosphate synthetase* *Glutaminase* *Rat liver mitochondria* *Ammonia* *Channeling* *Citrulline*

## 1. INTRODUCTION

According to [1] urea synthesis plays a central role in pH regulation of the body, because the synthesis of each molecule of urea is accompanied by production of one $H^+$ which can neutralize excess $HCO_3^-$ formed during protein catabolism. Indeed, in isolated hepatocytes urea synthesis from added ammonia is inhibited at low pH, although the sensitivity to small pH changes in the physiological range is not very high [2,3]. On the other hand, catabolism of glutamine is extremely sensitive to small pH changes [4], being inhibited at low pH, and for that reason presumably plays an important role in pH regulation [5]; this inhibition of glutamine degradation at low pH is due to a decreased affinity of mitochondrial glutaminase for its essential activator ammonia [6,7]. According to [5] simultaneous operation of glutaminase and carbamoyl-phosphate synthetase in periportal hepatocytes and of glutamine synthetase in perivenous hepatocytes has the net result of promoting urea synthesis which is necessary because of the relatively low affinity of urea synthesis for ammonia. In support of this view we have now obtained evidence which suggests that glutamine-derived ammonia is directly channeled to carbamoyl-phosphate synthetase. The results of these experiments are described here.

## 2. MATERIALS AND METHODS

Liver mitochondria were isolated from male Wistar rats (200–250 g) as in [6]. The rats were starved for 18 h and received a high-protein meal (80% casein) 1.5 h before killing in order to ensure high rates of citrulline synthesis. This procedure also results in a high activity of glutaminase.

Incubations were carried out at 30°C in closed 25-ml counting vials in a medium (3 ml) containing 25 mM 4-morpholinepropanesulfonic acid (Tris salt), 60 mM KCl, 1 mM EGTA, 10 mM potassium phosphate, 20 mM potassium succinate, 15 mM glutamine, 10 mM ornithine, 20 mM $KHCO_3$, 0.5 mM ATP, 0.15 mM $NH_4Cl$, 2 $\mu$g/ml rotenone and 50 mM mannitol (derived from the mitochondrial suspension); final pH 7.4. The gas phase was 95% $O_2$ + 5% $CO_2$. Incubations were terminated with $HClO_4$ (final concentration 3%, w/v) and neutralized as in [6].

Glutamate, ammonia and protein were measured as in [6]. Citrulline was determined according to [8].

## 3. RESULTS

In the experiment of fig.1 citrulline synthesis was studied with glutamine as the ammonia-donating substrate; succinate was present for

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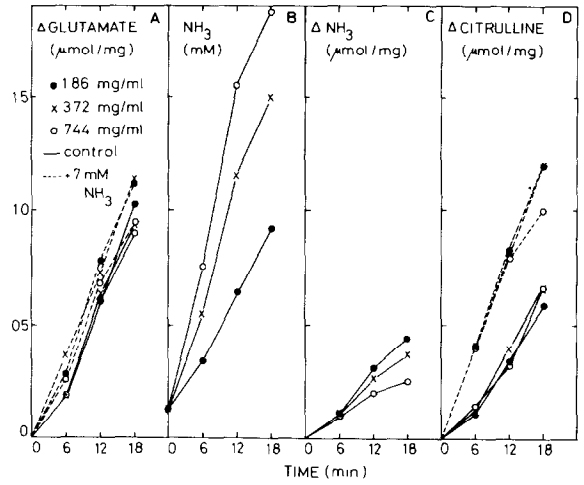


Fig.1. Citrulline synthesis from glutamine in isolated rat liver mitochondria. Mitochondria [(●) 1.86 mg protein/ml, (×) 3.72 mg protein/ml, (○) 7.44 mg protein/ml] were incubated as described in section 2. (——) Control, (– – –) 7 mM $NH_4Cl$ present. The experiment shown is representative of 4 analogous experiments that were carried out with 4 different mitochondrial preparations.

generation of ATP. Oxidation of glutamate was blocked by rotenone so that flux through glutaminase was equal to production of glutamate. At the start of the experiment a low concentration of ammonia (0.15 mM) was present to ensure full activation of glutaminase [6].

As shown in fig.1A flux through glutaminase, expressed per mg mitochondrial protein, was almost independent of the protein concentration used and was hardly affected by addition of excess $NH_3$ (7 mM), indicating that glutaminase was, indeed, fully active.

During incubation the concentration of ammonia in the suspension, which largely reflects that in the extramitochondrial fluid (not shown), increased with time and also with the protein concentration (fig.1B). Expressed per mg mitochondrial protein, production of ammonia was independent of the protein concentration at 6 min and decreased with increasing protein concentration after prolonged incubation (fig.1C).

Citrulline production, expressed per mg mitochondrial protein, was independent of the protein concentration used (fig.1D). This was unexpected because in the extramitochondrial fluid the concentration of $NH_3$, the substrate for carbamoyl-phosphate synthetase increased both with time and protein concentration. Carbamoyl-phosphate synthetase was not saturated with ammonia because addition of a large excess ammonia (7 mM) to the suspension doubled citrulline production at all protein concentrations (fig.1D). This effect was not due to a change in intramitochondrial pH because addition of 10 mM methylamine instead of ammonia did not affect citrulline production (not shown).

## 4. DISCUSSION

We have used glutamine as the intramitochondrial source of ammonia for carbamoyl-phosphate synthetase. Under these conditions, ammonia was efficiently used for citrulline synthesis since citrulline production was higher than ammonia accumulation.

It is well known, that the permeability of the mitochondrial inner membrane to $NH_3$ is high [9]. Although with time part of the ammonia produced in the glutaminase reaction leaked out of the mitochondria, carbamoyl-phosphate synthetase, which uses intramitochondrial ammonia, did not respond to the increasing concentration of ammonia in the medium despite the fact that the enzyme was not saturated with ammonia (fig.1D). This indicates that diffusion equilibrium of $NH_3$ across the mitochondrial inner membrane was not reached within the experimental period. Thus, ammonia generated by glutaminase was preferentially used for synthesis of carbamoyl phosphate rather than transported out of the mitochondria. The lag in citrulline synthesis (fig.1D) was probably due to the time required for the intramitochondrial ammonia concentration to reach a steady state. Indeed, with an excess of added ammonia the lag was no longer present (fig.1D). Because of channeling of ammonia from glutaminase to carbamoyl-phosphate synthetase, activation of glutaminase by ammonia indirectly increases the affinity of urea synthesis for ammonia. This is useful because ureogenesis has a relatively low affinity for portal ammonia [5]. With rising pH, which increases the affinity of glutaminase for ammonia [6,7] flux through this enzyme, and thus through the urea cycle, is accelerated.

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