# CHARACTERIZATION OF THE APPARENT RATES OF GLUTAMINE TRANSPORT IN RAT RENAL MITOCHONDRIA

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## 1. Introduction

Recent experiments have demonstrated the presence of a specific carrier system for transport of glutamine across the inner membrane of rat renal mitochondria [1,2]. Adaptation in this transport system could contribute to the regulation of the mitochondrial phosphate-dependent glutaminase activity and its participation in renal ammoniagenesis [3,4]. Mitochondria from acidotic and from potassium-depleted animals accumulated greater amounts of radioactivity when equilibrated with [$^{14}$C]glutamine, than did normal mitochondria [5–7]. The inability to detect glutamine within the matrix space, led to the conclusion that the increase in accumulation represented an adaptation in the transport process. Rat renal mitochondrial studies [8], purified of phosphate-independent glutaminase, have shown that significant amounts of glutamine are present within the matrix space and that glutaminase activity is probably the rate-determining step in rat renal metabolism of glutamine. An attempt to resolve these two processes is made here by analyzing the properties of mitochondrial glutamine transport at a reduced temperature.

## 2. Materials and methods

White male Sprague-Dawley rats (200–300 g) were obtained from Zivic-Miller and were fed Purina Rat Chow. Chronic metabolic acidosis was induced by providing the rats with a 0.28 M $NH_4Cl$ solution as their sole source of drinking water for periods of 2–10 days. L-[U-$^{14}$C]Glutamine, $^3H_2O$ and [U-$^{14}$C]-sucrose were obtained from New England Nuclear. [6,6'-$^3$H]Sucrose was a product of Amersham Searle. The silicone oil (Versilube F-50) was a product of General Electric Co. All other biochemicals were obtained from Sigma Chemical Co.

Rats were decapitated and their kidneys homogenized in 0.3 M sucrose, 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 1 mM EDTA solution, pH 7.4. Mitochondria were isolated using only differential centrifugation [9]. The phosphate-dependent glutaminase activity was determined by using glutamate dehydrogenase to quantitate the amount of glutamate formed [9]. Protein determinations were carried out as in [10]. Specific activities are reported in $\mu$mol $min^{-1}mg^{-1}$.

Glutamine transport studies were carried out in 400 $\mu$l centrifuge tubes and a Beckman model 154 microfuge, essentially as in [5]. The experiments were initiated by rapidly mixing 20 $\mu$l concentrated mitochondria with 100 $\mu$l cold transport buffer (4°C) which contained 1 mM glutamine, 130 mM KCl, 20 mM Hepes, 0.5 mM $MgSO_4$, 1 mM sodium arsenite and 1 $\mu$g/ml rotenone, pH 7.4. In some experiments, the mitochondria were preincubated with 1 mM unlabeled glutamine for 1 min at 24°C and quickly rechilled to 4°C before initiating the transport determination. After incubation in the transport buffer at 4°C for various times, the mitochondria were rapidly sedimented through 50 $\mu$l silicone oil into 50 $\mu$l 1.6 N perchloric acid. All experiments were performed using double labels and after centrifugation aliquots of the transport layer and of the perchloric acid layer were counted for radioactivity. In one set of experiments, [$^{14}$C]sucrose and $^3H_2O$ were added to the incubation

medium. From these determinations, the volumes of the outer space (space outside the inner mitochondrial membrane) and the total mitochondrial space were determined. The difference between these two volumes was considered to be the matrix space (space within the inner mitochondrial membrane). In a second set of experiments, [$^{14}$C]glutamine and [$^{3}$H]sucrose were present and these determinations provided a measure of uptake of label from glutamine and the volume of the outer space. It was assumed that label which was transported in excess of that required to equilibrate the outer space was concentrated in the matrix.

## 3. Results

At 24°C, the equilibration of radioactivity from [$^{14}$C]glutamine across the inner mitochondrial membrane is complete within 10–20 s [5,8]. The transport process is slowed considerably with decreasing temperature so that at 4°C, accumulation of label continues linearly for up to 20 min (fig.1). Mitochondria isolated from rats made acidotic exhibit increased phosphate-dependent glutaminase specific activity and increased apparent rates of glutamine transport. From the slopes of the data presented in fig.1, apparent transport rates of 0.008, 0.030 and 0.105 nmol min$^{-1}$mg$^{-1}$ were calculated for mitochondria with glutaminase specific activities of 0.12, 0.45, and 1.52 μmol min$^{-1}$mg$^{-1}$, respectively.

When the glutamine transported reaches 0.6 nmol mg$^{-1}$, the concentration of label within the matrix space is approximately equal to that in the transport medium. If the mitochondrial samples are incubated at 4°C for sufficiently long periods of time (40–60 min), the accumulation of label from [$^{14}$C]glutamine eventually plateaus at approximately the same values as reported for normal and acidotic mitochondria equilibrated at 24°C [5,8].

The apparent linear rates of accumulation of label from [$^{14}$C]glutamine within the mitochondrial matrix space do not extrapolate through the origin. As shown in fig.2, when mitochondria are rapidly centrifuged through the transport medium and into silicone oil in order to minimize the time of incubation (10–20s), significant label is still present within the matrix space. Preincubation of mitochondria with 1 mM unlabeled glutamine for 1 min at 24°C, followed by rapid cooling and determination of transport at 4°C, removes the initial high accumulation of label from [$^{14}$C]glutamine. But following such treatment, mitochondria from

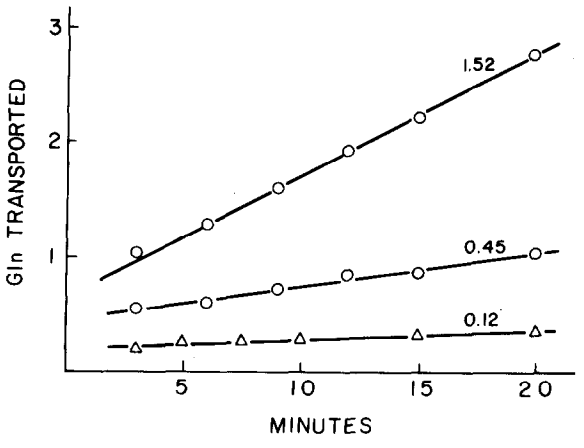


Fig.1. Determination of the rate of rat renal mitochondrial transport of glutamine at 4°C. Mitochondria were prepared from normal (triangles) and acidotic rats (circles) and they exhibited the indicated phosphate-dependent glutaminase specific activities. Glutamine (Gln) transported is reported as nmol of $^{14}$C-label accumulated/mg mitochondrial protein.

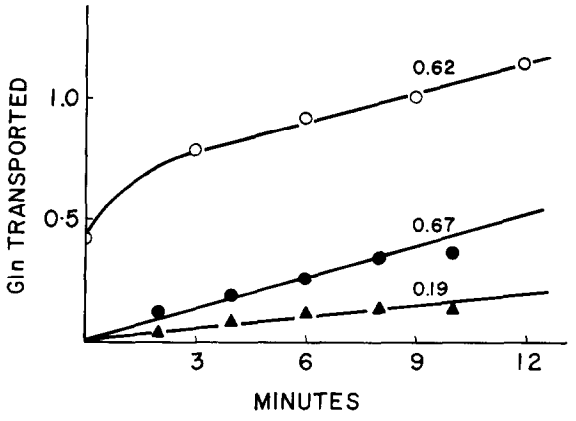


Fig.2. Effect of preincubation with glutamine on the rates of rat renal mitochondrial transport of glutamine at 4°C. Mitochondria were prepared from normal (triangles) and acidotic rats (circles) and exhibited the indicated phosphate-dependent glutaminase specific activities. Some samples were preincubated (closed figures) with 1 mM glutamine for 1 min at 24°C before measuring transport of [$^{14}$C]glutamine at 4°C. Glutamine (Gln) transported is reported as nmol $^{14}$C-label accumulated/mg mitochondrial protein.

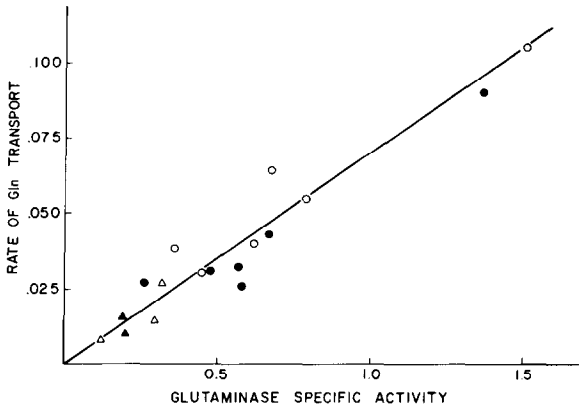


Fig.3. Comparison of the rate of mitochondrial glutamine (Gln) transport and the phosphate-dependent glutamine specific activity. Mitochondria were prepared from normal (triangles) and acidotic (circles) rats and some samples were subjected to preincubation (closed figures) with unlabeled glutamine before iniation of the transport determination. Rates of transport are given as nmol $^{14}$C-label accumulated/mg mitochondrial protein/min.

normal and acidotic rats still exhibit differences in apparent rates of glutamine transport. In addition, for mitochondria of similar phosphate-dependent glutaminase specific activities, the slope of the linear portion of the uptake profile is unaltered by preincubation.

The measured rates of uptake of label from [$^{14}$C]-glutamine from 17 different mitochondrial preparations are shown in fig.3. In order to compare samples of different glutaminase activities, mitochondria were prepared from normal rats and from rats made acidotic for various periods of time. In approx. 50% of the preparations, the mitochondria were subjected to preincubation with unlabeled glutamine. In all cases there is an excellent correlation between the increase in the apparent rate of glutamine transport and the phosphate-dependent glutaminase specific activity.

## 4. Discussion

The recent finding of glutamine within the matrix space of rat renal mitochondria indicates that in this species, glutamine is not hydrolyzed during the transport process [8]. It also suggests that the glutamine carrier and the phosphate-dependent glutaminase are distinct proteins. The data presented in this study is also consistent with this interpretation. As a result of reducing to 4°C, the overall process of accumulation of radioactivity from [$^{14}$C]glutamine within the mitochondrial matrix becomes biphasic. The initial phase of uptake probably reflects the rapid entry of glutamine into a matrix space depleted of glutamine. Whereas the slower and linear process of accumulation of radioactivity probably reflects conversion of glutamine to glutamate.

The observation that preincubation of mitochondria with unlabeled glutamine results in removal of the initial phase of uptake is consistent with the conclusion that this process represents the initial entry of glutamine into the matrix space. The rates of this process are extremely rapid. A large proportion of this process is complete even at 4°C within the minimum incubation time of this system. Therefore, resolution of the question as to whether there is an adaptation in the amount or the activity of the mitochondrial glutamine carrier during acidosis will require development of a more rapid system for analysis of transport.

The close correlations between the measured linear rates of accumulation of radioactivity and the phosphate-dependent glutaminase activity indicates that this process is a function of glutamine metabolism. From the observed profiles it would appear that this process is primarily responsible for the observed differences in fold accumulation of radioactivity from glutamine within normal and acidotic mitochondria. Therefore, the observed adaptations in rat renal mitochondrial glutamine transport [5,6], are probably due to the increased amounts of phosphate-dependent glutaminase and the resulting increase in glutamine metabolism. As a result, the previous studies should not be interpreted as an indication of an adaptation in rat renal mitochondrial glutamine transport system during acidosis.

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