DEACYLATION AND TRANSACETYLATION OF ACETYL GLUTAMATE AND ACETYL ORNITHINE IN RAT LIVER

A. REGLERO, J. RIVAS, J. MENDELSON, R. WALLACE and S. GRISOLIA
Dept. of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas 66103, USA
and *Instituto de Investigaciones Citologicas, Valencia, Spain*

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1. Introduction

Acetyl amino acids have been known to be metabolized by animals for nearly a century [1]. Also, the key role of acetyl glutamate in citrulline synthesis by animals [2] and for ornithine synthesis by micro organisms has been known for many years [3]. Greenstein et al. [4] purified from pig kidney a deacylase of relatively low specificity (EC 3.5.1.14) and another highly specific for acetyl aspartate (EC 3.5.1.15)*.

It is known that brain has acetyl aspartate deacylase as well as very high concentrations of acetyl aspartate [5] and that acetyl glutamate is present and synthesized within rat liver mitochondria; indeed, fairly wide and rapid fluctuations in levels of acetyl glutamate therein have been reported [6]. Thus, there must be acetyl glutamate deacylation or transacetylation in mitochondria and/or a mechanism to export it to the cytosol for degradation.

This communication presents a survey of *N*-acetyl-glutamate deacylase and transacetylase activities in rat liver mitochondria and some of the properties of the cytosol deacylase of rat liver. While a hitherto unsuspected marked activity with acetyl ornithine has been uncovered and weak acetyl glutamate deacylase and α-*N*-acetyl-ornithine L-glutamate *N*-acetyl transferase (EC 2.3.1.35), the latter activities are not of sufficient magnitude to explain the fluctation in acetyl glutamate levels of liver mitochondria, suggesting transport to the cytosol.

*It should be noted that the nomenclature is confusing; Greenstein [4] who discovered this enzyme called it acylase II. Other people refer to the enzyme and to the related acylase I of Greenstein as deacylases, others as amino-acylases and still others as *N*-acyl amino acid amido hydrolases. To avoid confusion, it should be remembered that acylase I is (EC 3.5.1.14) and acylase II is (EC 3.5.1.15); for simplification, we refer to them throughout this paper as deacylases (for the corresponding acetyl amino acid).

Adress correspondence to: Dr Santiago Grisolia, Instituto de Investigaciones Citologicas, Amadeo de Saboya, 4, Valencia-10, Spain

2. Materials and methods

Male Wistar rats, weighing from 250–300 g, were from Bio Lab. [^{14}C]-Glutamate was purchased from New England Nuclear, Boston, Mass. *N*-Acetyl L-amino acids, pyruvate kinase (Type III, lyophilized salt-free powder from rabbit muscle), lactate dehydrogenase (Type XI, lyophilized salt-free powder from rabbit muscle), NADH (disodium salt, Grade III) and ATP (disodium from equine muscle) were from Sigma Chemical Co, St Louis, Mo. Ninhydrin and Cellosolve were obtained from Pierce, Rockford, Ill. δ-Acetyl-ornithine was a gift from Dr R. Manske. All other chemicals were of analytical reagent grade.

Rats were killed by decapitation. Mitochondria and cytosol were prepared according to the method of Ragab et al. [7]. Mitochondria were washed three times with sucrose and two times with 0.15 M KCl. The packed mitochondria from 29 g liver were suspended in 2.5 ml water, broken with an Ultra-Turrax (20 s) and frozen and thawed once using a dry-ice–acetone bath and water at 20°C, respectively. They were then centrifuged at 11 000 × *g* for 5 min, the pellet extracted twice with 2.5 ml water and all supernatants combined. Two to three ml of the resulting supernatants were desalted on a Sephadex

G-25 fine column (2 X 32 cm), equilibrated and eluted with 0.02 M potassium phosphate buffer, pH 7.4.

Carbamyl phosphate synthetase was prepared as described by Nicoletti et al. [8] up to and including the $(NH_4)_2SO_4$ fractionation.

Unless specified otherwise, incubations were carried out at 37°C for 1 h in tightly covered tubes. Deacylase activity was assayed routinely by incubating in 1.0 ml, 50 μmol potassium phosphate buffer, pH 7.0, 5 μmol *N*-acetyl amino acid and the tissue preparation. Reactions were stopped with 1.0 ml 10% trichloroacetic acid. After 10 min on ice, the mixtures were centrifuged at 12 000 X *g* for 10 min in a Sorvall RC-2B. Supernatants were carefully removed with Pasteur pipettes and ninhydrin-positive material determined therein by the method of Spies [9]. Leucine was used as a standard. When measuring α-*N*-acetyl-ornithine deacylation, the reaction was carried out, at pH 9.6, using Tris–Cl^- buffer and 10 μmol α-*N*-acetyl-ornithine. Ornithine was determined in the soluble trichloroacetic acid supernatant by the ninhydrin method of Ratner [10].

Acetyl glutamate deacylase activity was assayed also by measuring acetate formation. In such instances, the reactions were stopped by heating at 100°C for 5 min. After centrifugation, the acetate was determined in the supernatant essentially as described by Kuo and Younathan [11], except that the pyruvate formed was determined colorimetrically by scaling down the volumes and reagents of the method of Friedemann and Haugen [12]. Acetyl glutamate was determined also by its stimulatory effect on carbamyl phosphate synthetase measured by a modification of the method of Chabas et al. [13] as follows: In a 1 cm cell, 0.15–0.3 units carbamyl phosphate synthetase [13] were used and up to 10 nmol acetyl glutamate. The assay was essentially linear at these low levels of acetyl glutamate. In all cases, the possible inhibition by the components of the samples was checked by adding acetyl glutamate. α-*N*-Acetyl-ornithine L-glutamate *N*-acetyl transferase was determined as follows: incubation mixtures contained in 1.0 ml, 50 μmol potassium phosphate buffer, pH 7.4; 20 μmol glutamate and, when used, 20 mM α-acetyl-ornithine or δ-acetyl-ornithine or 0.025–0.04 mM acetyl glutamate and 40 mM ornithine and approx. 3.5 mg mitochondrial protein. Incubation was for 2 h. The reaction was stopped by heating the tubes in a boiling water bath for 5 min. The acetyl glutamate was determined in the supernatant with carbamyl phosphate synthetase The transacetylase was also determined as follows: the reaction mixture contained in 50 μl, 10 μmol Tris–Cl^- buffer, pH 9.0, 0.5 μmol [^{14}C]glutamate containing 0.15 μCi, up to ∿1.3 mg mitochondrial protein, and, when used, 1 μmol α-*N*-acetyl-ornithine. The reaction was stopped with 20 μl 1 M HCl. Of the resulting mixture, 50 μl was passed through a small column of Dowex-AG 50 W in 10 mM HCl in order to absorb the [^{14}C]glutamate. Two μl 0.4 M non-radioactive *N*-acetyl-glutamate were used as carrier. The radioactive *N*-acetyl-glutamate formed passed through the column, and its radioactivity was measured in a scintillation counter. Protein was determined by a biuret method [14]. All measurements were corrected for endogenous values.

3. Results

As illustrated in table 1, high activity was observed with kidney and liver homogenates and a number of acetyl amino acids. Much less activity was detected in brain, spleen and heart. This is in agreement with previous results [15]. It is of interest that, on the basis of activity with *N*-acetyl-methionine, pig kidney has ∿50 times higher activity than rat kidny and human kidney has ∿40 times higher activity [16]. There are enough variations between the two tissues

Table 1
Hydrolysis rates of acetyl amino acids in rat kidney and liver homogenates

Substrate	Tissue	
	Kidney	Liver
Acetyl-methionine	246	152
Acetyl-glutamate	177	28
Acetyl-ornithine	61	23
Acetyl-aspartate	53	4
Acetyl-alanine	167	40
Acetyl-glycine	111	24
Acetyl-valine	89	26
Acetyl-cysteine	71	15

Activities are given in μmol/h/g tissue. Assays were carried out under the standard conditions (see text)

to indicate marked differences between the enzymes or the presence of isoenzymes as previously suggested [15].

When a liver homogenate in 0.15 M KCl was centrifuged at 10 000 × *g* for 30 min, >94% of the activity with *N*-acetyl-methionine or *N*-acetyl-ornithine was found in the cytosol.

Ornithine synthesis via *N*-acetyl-glutamate occurs in micro organisms. It is unknown whether such a pathway occurs in animal tissues; thus the activity with α-*N*-acetyl-ornithine in rat liver and kidney is of interest. Possibly the reason that this activity may have not been noted earlier in animal tissues is due to the alkaline optimum pH 9.6. As shown in fig.1, the activity is negligible at neutral pH. The α-*N*-acetyl-ornithine deacylase from *E. coli* shows optimal activity at pH 7.0 [17] and is stimulated by Co^{2+}. These ions in the range 1–10 mM had no stimulatory effect on the liver enzyme. As shown in fig.1 with acetyl methionine and with acetyl glutamate, the optimum activity is at neutral pH.

Using rat liver cytosol, desalted with Sephadex G-25, the K_m values, estimated by the procedure of Lineweaver and Burk [18], with acetyl glutamate, acetyl methionine and acetyl ornithine were 10 mM, 0.9 mM and 10 mM, respectively.

Acetyl glutamate synthetase is located in the matrix of rat liver mitochondria [6]. It makes, under optimal conditions, i.e., at 1 mM arginine, ∿400 nmol acetyl glutamate/g liver/h. Because of the rapid changes of levels of acetyl glutamate to caseine feeding and other conditions [6] and the recent reports of regulation of the urea cycle by levels of acetyl glutamate [19], it was of interest to search for acetyl glutamate deacylase activity in rat liver mitochondria. Due to proteases [20], we could not measure with

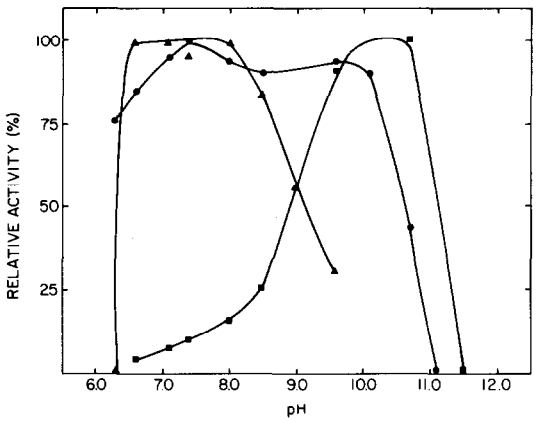

Fig.1. Effect of pH on the deacylase of rat liver cytosol. Activities were assayed with *N*-acetyl-methionine (●), *N*-acetyl-glutamate (▲) or *N*-acetyl-ornithine (■) as substrates, using phosphate buffer (pH 6.3–8.0 and pH 10.7–11.5), Tris–Cl^- (pH 7.7–9.6), glycine (pH 8.5–10.7) and carbonate (pH 9.6–10.7).

Table 2
Acetyl-glutamate deacylase and related activities of rat liver mitochondria

Enzyme activity	Activity (nmol)	Method/Ref.
Acetyl-glutamate synthetase	400	[24]
Acetyl-glutamate deacylase	< 1	Enzymatic[a]
Acetyl-glutamate deacylase	< 0.0	[11]
Transacetylase(s)		
α-acetyl-ornithine-glutamate[b]	∿ 1.0	Enzymatic[a]
α-acetyl-ornithine-glutamate[c]	0	Enzymatic[a]
δ-acetyl-ornithine-glutamate[b]	< 0.5	Enzymatic[a]
δ-acetyl-ornithine-glutamate[b]	< 0.03	Isotopic

[a]Enzymatic stimulation of carbamyl phosphate synthetase
[b]Estimated by measurement of acetyl glutamate formation
[c]Estimated by measurement of acetyl glutamate utilization

The concentration of acetyl glutamate in rat liver is ∿25 nM, but it can change with diet [6]. All data is given for 1 h incubation and per g equiv. liver. The deacylase activity was assayed using desalted (see text) mitochondrial extract corresponding to 0.9–1.3 g liver for 2 h with 0.025–20 mM acetyl glutamate

reliability low activity with ninhydrin. Therefore, we tested activity with acetyl glutamate in mitochondrial extracts by acetate production an by stimulation of carbamyl phosphate synthetase. Although as illustrated in table 2 there is some deacylase activity in mitochondrial extracts, it is much lower than the synthase.

Since α-*N*-acetyl-ornithine L-glutamate *N*-acetyl transferase could be responsible for acetyl glutamate utilization, we tested for this transacetylase (also for activity with δ-acetyl-ornithine) in rat liver mitochondria. As indicated in table 2, no or very low activity was detected.

4. Discussion

The deacylase activity of rat liver cytosol with α-acetyl-ornithine is of interest, but in view of the low activity for the transacetylase, it does not seem that rat liver posseses an ornithine synthetic pathway. Heat stability and fractionation experiments as well as competitive studies indicate that the activity with α-acetyl-ornithine is carried on by the deacylase I of Greenstein.

Although the significance of the fluctuation of acetyl glutamate levels reported by Tatibana [6] has been questioned in so far as a fine controller of urea synthesis [19,21], there is no doubt that the acetyl glutamate effect on carbamyl phosphate synthetase seems a key factor.

Of course, it appears important for the mitochondria of ureotelic animals to maintain a relatively high level of acetyl glutamate. Indeed, it seems from our data that the *N*-acetyl-glutamate cannot be degraded quickly enough in the mitochondria to account for the rapid variations in levels [6] within these particles by known pathways; therefore, there must exist some mechanism to transport this compound to the cytosol where it could be hydrolyzed. This is puzzling and most interesting, since reportedly acetyl glutamate does not enter mitochondria [22].

Since many proteins possess acetyl residues, the potent *N*-deacylases of the cytosol may also control the function of these proteins and/or serve as an agent permitting the further utilization of the acetylated amino acids. Indeed, it should be noted that an ε-acetyl-lysine deacylase presumably functions 'in vivo' to clear ε-acyl proteins [23].

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