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Methionine transamination *in vivo*

In a recent interesting paper, Wu & Thompson [1] presented evidence that the occurrence of a transamination pathway for the degradation of methionine in skeletal muscle should be seriously questioned. I agree with this conclusion. In my review article [2], cited by Wu & Thompson, I discussed the evidence both for and against the hypothesis. I pointed out in this review that the relative importance of the transamination pathway in methionine degradation must await further study although the transamination pathway does seem to be increased when the trans-sulphuration pathway is impaired [2]. Wu & Thompson [1] state that "This proposal [i.e. the transamination pathway] is largely based on the detection of methionine transamination in homogenates of skeletal muscle, liver and other tissues... and in extracts of rat skeletal muscle." They then criticize these studies because they were carried out at a concentration of methionine (10–20 mM) 100–200 times that of the plasma methionine concentration.

Reference to my work given by Wu & Thompson in this connection did not involve a measurement of the transamination between methionine and a 2-oxo acid [3]. In fact, I measured transamination between L-phenylalanine and 2-oxo-4-methylthiobutyrate (a measure of glutamine transaminase K activity) and between L-albizziin and 2-oxo-4-methylthiobutyrate (a measure of glutamine transaminase L activity). Both enzymes utilize 2-oxo-4-methylthiobutyrate as an excellent amine acceptor from glutamine.

Most transamination reactions are freely reversible; indeed, under appropriate conditions methionine can be shown to be a substrate for the glutamine transaminases (e.g. [4]). However, we have pointed out on several occasions (e.g. [3–6]) that glutamine is likely to be the amino acid substrate *in vivo*. Glutamine is generally present in tissues at a much higher concentration than methionine. Moreover, the reaction is forced in the direction of glutamine utilization because the product of glutamine transamination (2-oxoglutaramate) either cyclizes ($> 99\%$) or is deamidated to 2-oxoglutarate by ω-amidase.

In those tissues that contain high glutamine transaminase activity (e.g. liver, kidney) it seems unlikely that, under normal circumstances, 2-oxo-4-methylthiobutyrate formed by the transamination pathway can escape the tendency of the glutamine transaminases to redirect the carbon skeleton back to methionine. Theoretically, however, in those tissues with relatively low glutamine transaminase activity (e.g. skeletal muscle; [3, 6]) it may be possible for 2-oxo-4-methylthiobutyrate to accumulate, favouring catabolism of methionine through the transamination pathway. Moreover, the capacity of isolated mitochondria to convert 2-oxo-4-methylthiobutyrate to CO_2 (presumably via the branched-chain 2-oxo acid dehydrogenase complex [7, 8]) is considerable. Thus, it was of interest to read that Wu & Thompson have provided convincing evidence that the transamination pathway of methionine degradation may not occur in rat or chick skeletal muscle under normal circumstances. I would like to add a few more comments regarding this point.

Several studies of methionine metabolism *in vivo* in humans do not support the hypothesis of a major role for the transamination pathway in methionine degradation under normal circumstances. Gahl *et al.* [9] studied a 31-year-old patient with partial methionine adenosyltransferase (EC 2.5.1.6) deficiency. This patient has a 20–30-fold elevation of plasma methionine. Gahl *et al.* estimated that in this patient $\geqq 20\%$ of dietary methionine is catabolized by the transaminative pathway [9]. Evidently, even in this patient where the transaminative pathway will be favoured, the pathway cannot 'handle' excess methionine and methionine accumulates [9]. Gahl *et al.* also pointed out that patients with cystathionine synthase (EC 4.2.1.22) deficiency are grossly defective in their maximum capacity to convert methionine sulphur to sulphate (see [10, 11]). Furthermore, in a hypermethionaemic patient in whom the concentration of urinary 2-oxo-4-methylthiobutyrate was monitored, the daily output of this 2-oxo acid was negligible [10]. Martesson [12] has published a sensitive gas chromatography assay for 2-oxo-4-methylthiobutyrate and shown that very small amounts of this compound and its 2-hydroxy analogue are excreted daily in the urine of normal subjects. In a more recent study, gas-liquid chromatography was used to measure serum and urinary products of the methionine transamination pathway [13]. It was concluded that, in normal individuals, the transamination pathway (although it exists) is not a quantitatively important route for methionine breakdown, even after methionine loading [3].

In conclusion, methionine transamination does occur *in vivo*. Undoubtedly, the capacity of many tissues to transaminate methionine *in vitro* is considerable. However, there is much evidence to suggest that transamination of methionine *in vivo* is quantitatively not normally a major route for methionine degradation *in vivo*. The widespread activity of the glutamine transaminases in liver and kidney will ensure that 2-oxo-4-methylthiobutyrate, should it arise from non-specific transamination reactions or from catabolism of 5′-methylthioadenosine, will be largely salvaged as methionine.

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Methionine transamination and glutamine transaminases in skeletal muscle

The letter by Cooper [1] outlines additional support to the conclusion presented in our recent paper [2] that the physiological significance of a transamination pathway for methionine degradation in skeletal muscle should be seriously questioned. His letter also raises several issues to which we would like to respond.

We agree with Cooper [1] that glutamine transaminases K and L from rat liver and kidneys utilize 2-oxo-4-methylthiobutyrate as an excellent amine acceptor, and we have found that this is also true for rat but not chick skeletal muscle (Table 1). In their early studies, Cooper & Meister [3–5] demonstrated that glutamine transaminases K and L from rat liver and kidneys catalysed the transamination of many amino acids including 20 mM-methionine with α-oxo acids such as 20 mM-glyoxylate and 0.4 mM-phenylpyruvate. They also reported the transamination of 5 mM-2-oxo-4-methylthiobutyrate with 40 mM-L-albizziin in rat skeletal muscle, catalysed by glutamine transaminase L [5]. However, they did not measure the transmination of methionine itself with α-oxo acids in skeletal muscle extracts [5] as cited in our recent paper [2].

We have measured methionine transamination and glutamine transaminase K and L activity in skeletal muscles from rats and chicks (Table 1). Our results are not consistent with Cooper's theoretical concept that low glutamine transaminase activity in skeletal muscle may favour methionine degradation via a transamination pathway [1]. Although methionine is extensively transaminated in intact chick skeletal muscle incubated in the presence of 0.2–0.5 mM-methionine but in the absence of other amino acids [2], glutamine transaminase K and L activities are only barely detected in this tissue (Table 1). On the other hand, rat skeletal muscles contain considerable glutamine transaminase K and L activity, while methionine transamination is negligible or absent in intact muscles incubated in the absence of plasma concentrations of amino acids (Table 1). These results suggest that glutamine transaminases K and L do not play an important role in methionine degradation in skeletal muscle.

Table 1. Glutamine transaminase K and L activities and methionine transamination in rat and chick skeletal muscles

Mitochondrial and cytosolic fractions of skeletal muscles from 60–80 g fed Sprague–Dawley female rats and 115–130 g fed male broiler chicks (*Gallus domesticus*) were prepared as previously described [8]. Glutamine transaminase K was assayed in the presence of 10 mM-glutamine, 50 μM-phenylpyruvate and 50 mM-sodium borate (pH 8.5) as described by Cooper & Meister [3–5], except that phenylalanine was measured by h.p.l.c. [2]. Glutamine transaminase L was assayed in the presence of 10 mM-L-albizziin, 50 μM-2-oxo-4-methylthiobutyrate and 50 mM-sodium borate (pH 8.5) as described by Cooper & Meister [3–5], except that methionine was measured by h.p.l.c. [2]. Total activity of the enzyme is expressed as the cytosolic plus mitochondrial activity. Glutamine transaminase K and L activities in rat muscles are higher (*$P < 0.01$) than those in chick muscles as analysed by analysis of variance. Results are expressed as means ± S.E.M., $n = 3$. The methionine transamination data are obtained from reference [2]. N.D., not detectable.

Muscle	Glutamine transaminase K (nmol of Phe/h per mg of tissue)	Glutamine transaminase L (nmol of Met/h per mg of tissue)	Methionine transamination (nmol/2 h per mg of tissue)
Rat			
EDL	14.5 ± 0.1*	6.5 ± 0.6*	N.D.
Soleus	13.6 ± 0.1*	7.7 ± 0.1*	N.D.
Diaphragm	17.7 ± 0.1*	8.5 ± 0.1*	0.036 ± 0.003
Chick			
EDC	0.5 ± 0.02	0.4 ± 0.02	1.15 ± 0.08

It is clear from our studies that little or no 2-oxo-4-methylthiobutyrate is produced or accumulated in intact rat or chick skeletal muscles in the presence of plasma concentrations of amino acids [2]. Even though methionine is extensively transaminated in intact chick skeletal muscles incubated in the presence of 0.2–0.5 mM-methionine and in the absence of other amino acids, we found that only a very small amount of the 2-oxo-4-methylthiobutyrate produced undergoes oxidative decarboxylation [2]. These results suggest that the mitochondrial branched-chain 2-oxo acid dehydrogenase complex in skeletal muscle has very low activity towards 2-oxo-4-methylthiobutyrate, although this enzyme from bovine kidney cortex has been reported to catalyse the oxidative decarboxylation of 2-oxo-4-methylthiobutyrate to some extent [6].

Finally, we agree with Cooper [1] that transamination of methionine occurs in animals *in vivo* although it is normally not quantitatively a major route for methionine degradation. Additionally, Blom *et al.* [7] recently