Rate-limiting steps for protein synthesis in isolated rat liver cells

Role of aspartate availability

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Amino-oxyacetate (carboxymethoxylamine) was found to inhibit protein labelling in isolated liver cells. A similar degree of inhibition (about 70%) was observed of basal and substrate-stimulated rates of protein labelling, ruling out an action on the cellular energy state. Its effect does not seem to be related either to a perturbation of the reduction state of the NAD system or to rate changes in the gluconeogenic pathway. The following observations indicate that amino-oxyacetate inhibits protein labelling by limiting aspartate supply. (1) Amino-oxyacetate was ineffective in a postmitochondrial supernatant under non-limiting amino acid supply conditions. (2) The aspartate cellular content decreases in the presence of amino-oxyacetate, although most other amino acids tend to accumulate. (3) L-Cycloserine was unable to decrease aspartate content and was ineffective in decreasing protein labelling. (4) The inhibitory action of amino-oxyacetate was specifically reversed by incubating cells with amino acids that increase the cellular content of aspartate.

INTRODUCTION

Amino-oxyacetate (carboxymethoxylamine), a well-characterized inhibitor of pyridoxal-dependent enzymes [1,2], has also been described to be an effective inhibitor of protein synthesis in liver cells [3,4]. Amino-oxyacetate also displays a wide variety of metabolic inhibitory effects in liver. A shift of the cytosolic NAD system to a more reduced state [5], probably secondary to an inhibition of the malate–aspartate shuttle [1,5–8], seems to be a primary effect of amino-oxyacetate, leading to the perturbation of other metabolic pathways [1,8]. The state of reduction of the NAD system has been claimed to influence protein synthesis [9]. Thus amino-oxyacetate offered a unique tool with which to explore this possibility further. Our current work is an attempt to elucidate the mechanisms implicated in the inhibition of liver protein synthesis by amino-oxyacetate. We have considered two possibilities: (a) that this inhibitor acted directly, competing with some amino acid or limiting some pyridoxal-dependent reaction involved in protein synthesis; (b) an indirect mechanism could be a perturbation of other metabolic pathways, leading to changes in nucleotide content and/or the availability of some limiting amino acids. Our results seem to indicate that amino-oxyacetate exerts its inhibitory effect on liver cells primarily by limiting aspartate availability.

EXPERIMENTAL

Animals

Male Wistar albino rats weighing 200–220 g were used. The animals were starved for 48 h before the experiment was started.

Chemicals

[U-³H]Valine (10 Ci/mol) was purchased from Amer-

sham International (Amersham, Bucks., U.K.). All the reagents were of the highest possible purity, and most of them were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Collagenase was purchased from Worthington (Freehold, NJ, U.S.A.), and the other enzymes and liver tRNA were from Boehringer (Mannheim, Germany). L-Cycloserine was a gift from Dr. W. E. Scott of Hoffmann–La Roche (Nutley, NJ, U.S.A.).

Isolation and incubation of rat liver cells

The procedure for isolation of liver cells was described previously [4]. The cells were incubated in Krebs–Ringer bicarbonate buffer containing 2% Ficoll 70. The pH of the incubation mixture was 7.4 after equilibration with O₂/CO₂ (19:1).

Determination of protein-synthesis activity

L-[U-³H]Valine was utilized as the amino acid precursor, since it is poorly oxidized or transaminated by the liver cells [10]. Routinely, 1 ml portions of liver cells (40–60 mg wet wt./ml) were placed in plastic 25 ml Erlenmeyer flasks and incubated at 36.5 °C in a rotary shaker. The incubation medium contained 0.5 mM-L-[³H]valine (sp. radioactivity 10 Ci/mol). This concentration seems to be close to saturation [4], thus minor changes in the size of the valine pool utilized for protein synthesis would not induce appreciable changes in the rate of protein labelling. At the end of the experiment, triplicate samples (0.1 ml) were immediately added to cold 10% trichloroacetic acid and processed as described previously [4].

Determination of the gluconeogenic rate and state of reduction of the NAD system

For these determinations, the cell incubation medium contained 1.5% gelatin instead of Ficoll 70, which is hydrolysed at low pH, yielding free glucose. Routinely 2–3 ml of cell suspension (40–60 mg wet wt./ml) was

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incubated in plastic 25 ml Erlenmeyer flasks at 36.5 °C with constant rotary shaking; portions (1 ml) of the cell suspensions were taken after 1 h of incubation and immediately acidified with cold HClO_4 (final concn. 7%, v/v). The precipitate was sedimented by centrifugation at 13000 rev./min for 2 min in an Eppendorf micro-centrifuge and the supernatant neutralized to pH 7.0 with 3 M-KOH containing 0.5 M-triethanolamine. The samples were centrifuged at 13000 rev./min for 2 min, and glucose, lactate and pyruvate were determined by previously described enzymic procedures [11].

Determination of the intracellular amino acid content of the liver cells

For this determination 2 ml portions of the cell suspension in KRB Ficoll 70 medium were incubated at 36.5 °C in a rotary shaker. Samples (0.8 ml) were taken at zero time and after 1 h of incubation and immediately centrifuged at 13000 rev./min for 30 s in an Eppendorf micro-centrifuge. The supernatant was then discarded and the pellet resuspended in 0.8 ml of cold 7% HClO_4 . After removal of the acid-insoluble material by centrifugation, the samples were adjusted to pH 2 by addition of 1 M-lithium citrate, pH 1.3. The concentrations of the different amino acids were determined by ion-exchange liquid chromatography.

Determination of the protein-synthesis rate by postmitochondrial supernatants

For these experiments, small liver biopsies were taken from anaesthetized rats. Postmitochondrial supernatants were prepared as described previously [12].

The incubation mixture (final volume 0.5 ml) contained: 2 mM-ATP, 0.8 mM-GTP, 3 mM-magnesium acetate, 150 mM-KCl, 5 mM-Tris/HCl, pH 7.8, 0.1 mM

concentrations of all the natural amino acids except valine, 0.02 mM-[U- ^3H]valine (10 Ci/ml), 2 mM-phosphoenolpyruvate, 20 mg of pyruvate kinase/ml, and 2–2.25 mg of postmitochondrial-supernatant protein. After incubation at 37 °C for 5 min, 100 μl samples were taken; 2 ml of 10% trichloroacetic acid and 1 mg of bovine serum albumin were then added to each. After heating at 90 °C for 15 min, the samples were filtered through Millipore membranes, each sample being washed with 5 $\times$ 2 ml of 5% trichloroacetic acid each time. The filters were dried and placed in vials with 15 ml of a toluene/2,5-diphenyloxazole mixture.

The protein content of the postmitochondrial supernatants was determined by the Lowry method [13].

The glutamyl-tRNA synthetase activity was determined in pH 5 supernatants obtained from rat liver as previously described [14]. The reaction mixture (1 ml final volume) contained 50 μmol of Hepes, pH 7.5, 10 μmol of MgCl_2 , 1.0 μmol of EDTA, 10 μmol of mercaptoethanol, 10 μmol of ATP, 10 μmol of CTP, 5 μmol of phosphoenolpyruvate, 1 μg of pyruvate kinase, 0.1 mg of liver tRNA, 1 μCi of [^{14}C]glutamate and 0.1 ml of pH 5 supernatant. The incubation was performed at 30 °C, and the reaction rate was linear for 30 min.

The aspartyl-tRNA synthetase activity of liver extract was determined in the 150000 g pellet obtained as described by Geels *et al.* [15]. The reaction mixture (final volume 0.125 ml) contained: 6.25 μmol of triethanolamine, pH 7.5, 6.25 μmol of KCl, 0.25 μmol of dithiothreitol, 0.5 μmol of ATP, 1 μmol of magnesium acetate, 0.125 μmol of CTP, 0.0125 mg of liver tRNA, 1 μCi of [^{14}C]aspartate and 40 μl of 150000 g pellet. The incubation was performed at 30 °C and proceeded in a linear fashion for at least 15 min.

Fig. 1. Effect of amino-oxacetate on rates of protein labelling in isolated liver cells

Liver cells were incubated in the absence of any substrate (a) or in the presence of either 10 mM-glucose (b) or 10 mM-alanine (c) and the indicated concentrations of amino-oxacetate. The rate of protein labelling was taken for the 30–60 min interval.

Table 1. Effect of an amino acid mixture on the inhibition by amino-oxyacetate of protein labelling

Liver cells were incubated in the presence and in the absence of an amino acid mixture that contained 1 mM of all the amino acids but valine. The rate of protein labelling was taken for the 30–60 min interval. The results are mean values $\pm$ S.E.M. for four different experiments: * $P < 0.05$ by t test.

Addition . . .	None		Amino acid mixture	
	(c.p.m./h per mg wet wt.)	(% inhibition)	(c.p.m./h per mg wet wt.)	(% inhibition)
Control	312 $\pm$ 33	–	565 $\pm$ 49	–
Amino-oxyacetate (0.2 mM)	150 $\pm$ 15*	52	500 $\pm$ 40	12

RESULTS AND DISCUSSION

Effect of amino-oxyacetate on rates of protein labelling by isolated liver cells

The results presented in Fig. 1 indicate that, whether basal (panel *a*) or substrate-stimulated rates (panels *b* and *c*) of protein labelling are considered, amino-oxyacetate displays similar dose-dependent inhibitory effects. Maximal inhibitory effects attained under all conditions were similar. Since protein labelling was not fully prevented, even at the highest dose of the inhibitor tested, this observation may indicate that a percentage of the total protein-synthesis activity, about 30%, is insensitive to amino-oxyacetate.

Under our experimental conditions, with protein- and amino-acid-free incubation medium, the concentration of amino-oxyacetate required to produce 50% inhibition is about 20 μ M. This observation contrasts with the 2 mM concentration described by Seglen *et al.* [3]. A non-specific

binding of the inhibitor to some of the incubation-medium components, perhaps albumin, or the competitive action of some of the metabolites with which the medium was supplemented, might explain the difference. Our observation that amino-oxyacetate is fully effective in inhibiting protein labelling in micromolar concentrations is more in accordance with the reported K_i for aspartate and other aminotransferases [16] and also with the concentrations described to lead to several metabolic perturbations [1,4–7]. Thus our results suggest that the inhibition of protein labelling by amino-oxyacetate may be related to its ability to decrease flux through certain key aminotransferases. Further support for this interpretation may be found in Table 1, which illustrates the reversibility of the amino-oxyacetate action on protein labelling by a mixture of amino acids. Although these data point to the participation of some amino-oxyacetate-sensitive aminotransferase reaction in its effect inhibiting protein labelling, it cannot be discerned whether its action

Table 2. Effect of amino acids on protein labelling in the presence and in the absence of amino-oxyacetate

Liver cells were incubated for 1 h in the presence of the indicated amino acids at a concentration of 5 mM.

Amino acid added	Control		Amino-oxyacetate (0.2 mM)	
	(c.p.m./mg wet wt.)	(% stimulation)	(c.p.m./mg wet wt.)	(% stimulation)
None	967	–	496	–
Leucine	999	3	431	–
Isoleucine	914	–	448	–
Alanine	1792	85	575	16
Proline	1397	44	1200	142
Glycine	1000	3	505	2
Methionine	1261	30	586	18
Cysteine	1177	22	462	–
Histidine	1353	40	597	20
Lysine	1481	53	524	6
Arginine	1075	11	319	–
Tryptophan	1274	32	433	–
Phenylalanine	1235	28	463	–
Serine	1006	4	544	10
Threonine	953	–	455	–
Glutamate	1212	25	483	–
Glutamine	1091	13	908	83
Aspartate	1091	13	588	18
Asparagine	1332	38	1085	118
Tyrosine	1203	24	415	–
Ornithine	1117	15	642	29

is exerted on some pyridoxal-dependent reaction involved in protein synthesis, or is mediated by an increased NAD redox state and/or altered flux through other metabolic pathways secondary to inhibition of aspartate aminotransferase. These possibilities have been further explored experimentally.

Effect of individual amino acids on the inhibitory effect of amino-oxyacetate on protein labelling

Since the presence of an amino acid mixture prevented the effect of amino-oxyacetate on protein labelling (Table 1), it was decided to determine whether specific individual amino acids related to the aspartate aminotransferase reaction were effective, or whether it was a non-specific effect related to substrate supply and/or increased energy production. According to data in Table 2, only proline, glutamine and asparagine were effective in increasing the rate of protein labelling in the presence of amino-oxyacetate. These amino acids share the capacity to increase the intracellular concentration of glutamate and/or aspartate [17–19]. Since the inhibitory effect of amino-oxyacetate on aspartate aminotransferase is a function of the concentration of those two amino acids when tested in the isolated purified enzyme as well as in the intact cell [20], the results in Table 2 seem to indicate that normal flux through aspartate aminotransferase is required in order to maintain normal rates of protein labelling. The lack of response to aspartate is not surprising, in view of the low rate of metabolic conversion of this amino acid [21], which indicates that its permeability across the plasma membrane is highly restricted. Thus, at the concentration of the amino acid used in these experiments, its rate of metabolic conversion seems to be similar to the rate of transport, resulting in no detectable changes in the cytosolic steady-state concentration.

Relationship between metabolic perturbations induced by amino-oxyacetate and its effect on protein labelling

When amino-oxyacetate was tested on a liver postmitochondrial supernatant, no detectable effects

were observed on rates of protein labelling (results not shown). This finding suggests that, by whatever mechanism its action is mediated, it does not involve a direct interaction with the protein-synthesis machinery. Furthermore, amino-oxyacetate was not able to inhibit glutamyl- or aspartyl-tRNA synthetase when assayed at a concentration of 0.2 mM in the presence of tracer amounts of the amino acid (results not shown).

On these grounds, it was decided to study whether the effect on protein synthesis was related to the metabolic perturbations induced by the inhibitor. For this purpose a comparative study with the action of cycloserine, another inhibitor of pyridoxal-dependent reactions [22–27], was carried out. Fig. 2 shows the striking difference between the effect of both aminotransferase inhibitors on protein labelling in liver cells in the presence of gluconeogenic precursors of different reduction states. Although amino-oxyacetate was effective regardless of the substrate utilized, extremely large concentrations of cycloserine were required to inhibit protein labelling only in the presence of lactate. The latter effect, in view of the concentrations of cycloserine required, several orders of magnitude above the reported K_i for the aminotransferase [26], can probably be explained by taking into account the reported action of cycloserine in decreasing pyruvate availability [28]. The differential effect of these two inhibitors on protein synthesis cannot be explained on the basis of a lack of permeability of the mitochondrial membrane towards L-cycloserine, since it has been shown that L-cycloserine effectively inhibits alanine metabolism in isolated mitochondria [29]. Alternative explanations could be that L-cycloserine is a less effective inhibitor of aspartate aminotransferase than is amino-oxyacetate [7,29], or transaminase activity is not essential for protein synthesis.

The inhibition of the malate–aspartate shuttle by aminotransferase inhibitors leads to a more reduced cytosolic NAD system and inhibition of gluconeogenesis from reduced precursors such as lactate [6,7,30,31]. Thus the experimental design utilized in the experiments

Fig. 2. Effect of amino-oxyacetate and cycloserine on rates of protein labelling in isolated liver cells incubated in the presence of 10 mM-lactate (●) or 10 mM-pyruvate (○)

Liver cells were incubated in the presence of the indicated concentrations of amino-oxyacetate or cycloserine. The rate of protein labelling was taken for the 30–60 min interval. The results are average values of six experiments and the vertical bars show S.E.M.

Fig. 3. Effect of amino-oxyacetate and cycloserine on rates of glucose production by isolated liver cells

Liver cells were incubated in the presence of either 10 mM-lactate (●) or 10 mM-pyruvate (○) and the indicated concentrations of amino-oxyacetate and cycloserine. The results are average values of four different experiments and the vertical bars show S.E.M.

shown in Fig. 2 allowed us to test simultaneously the influence of rather large variations in the cytosolic redox state and gluconeogenic flux on the rate of protein labelling. As shown in Figs. 3 and 4, under our experimental conditions both aminotransferase inhibitors displayed similar effects on those parameters: shift of the cytosolic NAD system toward a more reduced state, as indicated by the rise in the lactate/pyruvate ratio, and inhibition of gluconeogenesis only from a reduced substrate such as lactate.

The lack of correlation between the observed metabolic perturbations and the effect on protein labelling allows us to conclude that those parameters are not involved in the amino-oxyacetate action. The observation that normal rates of gluconeogenesis can be attained (Fig. 3) in the presence of amino-oxyacetate rules out the possibility that the observed inhibition of protein labelling could be the result of a perturbation of cellular energy metabolism. This conclusion is also supported by reports indicating that amino-oxyacetate does not induce detectable

Fig. 4. Effect of amino-oxyacetate and cycloserine on the lactate/pyruvate ratios in isolated liver cells

Liver cells were incubated in the presence of either 10 mM-lactate (●) or 10 mM-pyruvate (○) and the indicated concentrations of amino-oxyacetate and cycloserine. The lactate/pyruvate ratio was determined after 60 min of incubation. The results are average values of three different experiments.

Fig. 5. Effect of amino-oxyacetate on rates of protein labelling in isolated liver cells

Liver cells were incubated in the absence or in the presence of 5 mM of the following amino acids: none (a), asparagine (b), proline (c) and glutamine (d), and in the absence (●) or in the presence (○) of 0.2 mM-amino-oxyacetate. The results are average values of five different experiments and the vertical bars show S.E.M.

changes in the rate of cellular oxygen uptake [5] or ATP content [7].

Correlation between cellular amino acid content and ability of amino-oxyacetate to inhibit protein labelling

The evidence presented so far seems to suggest that some event dependent on flux through aspartate aminotransferase is responsible for the inhibitory effect of amino-oxyacetate on protein labelling. However, the observation that amino-oxyacetate had no effect on protein labelling in a postmitochondrial supernatant in the presence of unlimited supply of amino acids, considering also the lack of effect of L-cycloserine in intact cells, clearly indicates that amino-oxyacetate does not act directly on the protein-synthesis machinery, but probably indirectly by limiting amino acid supply. Two observations strongly suggest that it is not the overall amino acid supply, but the availability of some key amino acid reactant of the aspartate aminotransferase, that may be limiting in the presence of amino-oxyacetate. First, the measurement of the intracellular concentration of amino acids indicates that most of them, the only

exceptions being aspartate and the aromatics, accumulated in the presence of amino-oxyacetate (Table 3). Second, the inhibitory effects of amino-oxyacetate were overcome by the addition of single amino acids whose metabolism leads to glutamate and/or aspartate formation (Table 2 and Fig. 5). These data strongly suggest that the decrease in aspartate availability might play an important role in the amino-oxyacetate action. On these grounds, it was decided to determine the effect of amino-oxyacetate on the cellular concentration of aspartate and glutamate under several incubation conditions. The results are summarized in Table 4. The interest of these data is threefold: first, in contrast with amino-oxyacetate, L-cycloserine had no effect in decreasing aspartate concentration; second, in the presence of asparagine, glutamine or proline, amino acids that effectively reversed the amino-oxyacetate-induced inhibition (Table 4), glutamate and aspartate concentrations were significantly increased, and amino-oxyacetate either had no effect in decreasing aspartate, or, if it did, the concentration remained above the basal control value; third, ornithine and histidine induced a significant

Table 3. Effect of amino-oxyacetate on the intracellular concentrations of amino acids

Liver cells were incubated for 1 h in the absence of any substrate. The results are mean values $\pm$ S.E.M. for six different experiments: * $P < 0.01$ by t test.

	Concn. (μ mol/g wet wt.)	
	Control	Amino-oxyacetate (0.2 mM)
Aspartate	0.48 ± 0.06	$0.27 \pm 0.03^*$
Threonine	0.42 ± 0.05	0.88 ± 0.07
Serine	0.44 ± 0.05	0.77 ± 0.07
Glutamate	0.98 ± 0.24	$2.47 \pm 0.41^*$
Glutamine	0.38 ± 0.05	0.37 ± 0.04
Glycine	2.80 ± 0.45	3.53 ± 0.55
Alanine	0.18 ± 0.04	0.94 ± 0.13
Valine	0.31 ± 0.04	0.48 ± 0.10
Methionine	0.38 ± 0.07	0.42 ± 0.10
Isoleucine	0.17 ± 0.03	0.21 ± 0.05
Leucine	0.29 ± 0.04	0.30 ± 0.08
Tyrosine	0.36 ± 0.07	0.33 ± 0.09
Phenylalanine	0.66 ± 0.08	$0.33 \pm 0.07^*$
Ornithine	0.20 ± 0.03	$0.14 \pm 0.04^*$
Lysine	0.24 ± 0.03	0.28 ± 0.05
Histidine	0.21 ± 0.02	0.25 ± 0.04
Total	8.5	11.97
Ammonia	4.64 ± 0.80	4.14 ± 0.63

increase in glutamate, but not in aspartate (Table 4), and were not able to relieve the inhibitory effect of amino-oxyacetate on protein synthesis (Table 2). Thus an apparent correlation seems to exist between the ability of amino-oxyacetate to decrease aspartate availability and to decrease protein labelling.

The incomplete inhibitory action of amino-oxyacetate

on protein labelling in liver cells is also compatible with the possibility that its action is mediated through a decreased aspartate concentration. Amino-oxyacetate displaces the equilibrium position of the reactants toward a decreased aspartate concentration, but of course it cannot make it approach zero concentration. Aspartate is also provided by cellular proteolysis in amounts probably sufficient to prevent a full inhibitory effect.

It should be remarked that the concentration of aspartate found in liver cells (Table 2) is one-tenth of that found in livers of fed intact animals and one-fifth of that found in 48–72 h-starved rats (results not shown). Not only aspartate, but virtually all amino acids, are decreased in content in freshly prepared isolated liver cells. Some authors have emphasized previously the need to supplement the incubation medium with certain amino acids in order to attain normal metabolic rates [32].

The intracellular distribution of aspartate has been a subject of controversy. Mitochondrial/cytosolic gradients from 0.2 to well above unity have been described [33,34]. Assuming an even distribution of aspartate and cytosolic and mitochondrial water contents of 2.0 and 0.2 ml/g dry wt. [35], then the cytosolic aspartate concentration reached in our experiments would be 0.7 mM. Although high K_m values for aspartate have been reported for certain aspartyl-tRNA synthetases [36], in mammalian tissues the K_m for amino acids of the aminoacyl-tRNA synthetases seems to be in the micromolar range [37]. Actually, under our experimental conditions the K_m for aspartyl-tRNA synthetase was below 100 μ M (results not shown). Thus the observation that a decrease in cytosolic aspartate below 0.7 mM causes a decreased rate of protein labelling under conditions under which all other amino acids accumulate suggests the following explanations: first, the K_m for aspartate of the isolated aspartyl-tRNA synthetase differs from that

Table 4. Effect of asparagine, proline and glutamine on the intracellular contents of aspartate and glutamate in the absence and in the presence of amino-oxyacetate

Liver cells were incubated in the presence of 5 mM concentrations of the indicated amino acids. The results are mean values $\pm$ S.E.M. for the numbers of experiments in parentheses. Statistical evaluation of the data was by the t test, by comparing each group with the control group in the absence of inhibitors: * $P < 0.001$; ** $P < 0.01$; *** $P < 0.02$; **** $P < 0.05$.

	Amino-oxyacetate (0.2 mM)	Cycloserine (10 mM)	Content (μ mol/g wet wt.)	
			Aspartate	Glutamate
Control (8)	—	—	0.48 ± 0.06	0.98 ± 0.24
	+	—	$0.27 \pm 0.03^{**}$	$2.47 \pm 0.41^{**}$
	—	+	$0.70 \pm 0.09^{****}$	1.05 ± 0.20
Asparagine (4)	—	—	$1.97 \pm 0.28^*$	$3.08 \pm 0.83^{***}$
	+	—	$2.05 \pm 0.10^*$	$3.80 \pm 1.13^{***}$
Proline (4)	—	—	$0.83 \pm 0.14^{***}$	$10.37 \pm 2.79^{**}$
	+	—	$0.78 \pm 0.14^{****}$	$18.10 \pm 4.75^*$
Glutamine (5)	—	—	0.67 ± 0.18	$6.45 \pm 1.48^*$
	+	—	0.49 ± 0.11	$17.55 \pm 3.65^*$
Ornithine (4)	—	—	0.63 ± 0.18	$2.66 \pm 0.31^{**}$
	+	—	$0.27 \pm 0.04^{****}$	1.35 ± 0.19
Histidine (4)	—	—	0.73 ± 0.25	1.30 ± 0.27
	+	—	$0.26 \pm 0.03^{****}$	$4.44 \pm 0.94^{**}$

of the enzyme located in its local microenvironment; second, the aspartate measured in acid-soluble extracts is an accurate indication of its total content, but by no means indicates the actual concentration of the metabolically active free amino acid, suggesting that an important proportion of the amino acid may not be accessible to the tRNA synthetase as a result of binding and/or compartmentation.

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