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Regulation of 2-Oxoglutarate Metabolism in Rat Liver by NADP-Isocitrate Dehydrogenase and Aspartate Aminotransferase

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Abstract—Kinetic and regulatory properties of NADP-isocitrate dehydrogenase (NADP-IDH) and aspartate aminotransferase (AsAT) responsible for 2-oxoglutarate metabolism in the cytoplasm and mitochondria of rat liver were studied. Based on the subcellular location of these enzymes and their kinetic parameters (K_m, K_{si}) obtained with highly purified enzyme preparations, it is suggested that synthesis of 2-oxoglutarate should be mainly determined by cytoplasmic NADP-IDH (86% of the total activity in the cell), whereas its utilization should depend on cytoplasmic AsAT (78% of the total activity). AsAT from the rat liver was specified by substrate inhibition and also by changes in the enzyme affinity for the substrates under the influence of some intermediates of the tricarboxylic acid cycle: isocitrate, succinate, fumarate, and citrate. Key intermediates of nitrogen metabolism (glutamate, glutamine, and aspartate) are involved in the regulation of NADP-IDH and AsAT. These enzymes are regulated oppositely, and the catalytic activity of one enzyme can be stimulated concurrently with a decrease in the activity of the other. Obviously, carbon and nitrogen metabolism in the rat liver can be controlled through redistribution of 2-oxoglutarate between different metabolic processes via regulatory mechanisms influencing differently located forms of NADP-IDH and AsAT.

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Nitrogen metabolism is closely associated with carbon metabolism because intermediates of glycolysis, the tricarboxylic acid cycle (TAC), and the pentose phosphate pathway are the main sources for carbon skeletons of amino acids. 2-Oxoglutarate is thought to be the most important intermediate providing the carbon skeletons for biosynthesis of amino acids because it is the most active intermediate acceptor of amino groups [1, 2]. The rates of nitrogen metabolism reactions depend on transamination with this metabolite.

Enzymes responsible for synthesis of 2-oxoglutarate are located in different cellular compartments, and it is still not known which enzymatic reaction is crucial in 2-oxoglutarate synthesis in animal and plant cells. Reactions catalyzed by NADP-isocitrate dehydrogenase (NADP-IDH, EC 1.1.1.42) and aspartate aminotransferase (AsAT, EC 2.6.1.1) are believed to play the main role [2-4]. There are many data on the structure and catalytic properties of NADP-IDH and AsAT [5-9], but their involvement in coordination of carbon and nitrogen metabolism through regulation of 2-oxoglutarate transformations in different subcellular compartments remains unclear. Data on the regulation of the NADP-IDH and AsAT activities are insufficient; therefore, there are no concepts about mechanisms for coordination of functioning of these enzymes, but such mechanisms are likely to exist.

The purpose of the present work was to comparatively characterize kinetic and regulatory properties of differently located forms of NADP-IDH and AsAT from rat liver to reveal possible mechanisms responsible for coordination of functioning of these enzymes. This is essential for understanding the coupling and regulation of the metabolic pathways of carbon and nitrogen.

Abbreviations: NADP-IDH) NADP-isocitrate dehydrogenase; AsAT) aspartate aminotransferase; SDH) succinate dehydrogenase; LDH) lactate dehydrogenase; TAC) tricarboxylic acid cycle.

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MATERIALS AND METHODS

Male white rats (*Rattus rattus* L.) with body weight of 250-300 g were used. The animals were maintained in a vivarium on a standard diet. The liver to be studied was isolated under anesthesia after a prolonged perfusion with ice-cold saline [10].

The enzyme activities were determined spectrophotometrically at $\lambda = 340$ nm. The reaction medium for determination of the NADP-IDH activity contained 0.05 mM Tris-HCl buffer (pH 7.8), 1.5 mM isocitrate, 2 mM $MnCl_2$, and 0.3 mM NADP. The rate of the IDH reaction was assessed by the increase in the optical density caused by NADP reduction.

The rate of the forward transamination catalyzed by AsAT was assessed by the decrease in optical density as a result of NADH oxidation in the system of coupled enzymatic reactions which included the production of oxaloacetate under the influence of AsAT and its subsequent transformation under the influence of malate dehydrogenase [11, 12]. The activity of AsAT was determined in 0.05 M potassium phosphate buffer (pH 7.5) containing 0.75 or 1.5 mM L-aspartate (for cytoplasmic or mitochondrial AsAT, respectively), 2.5 or 3.5 mM 2-oxoglutarate (for cytoplasmic or mitochondrial AsAT, respectively), 0.1 mM NADH, and malate dehydrogenase (1 U/ml).

The rate of the back transamination catalyzed by AsAT was assessed by the decrease in optical density as a result of NADPH oxidation in the system of coupled enzymatic reactions which included the generation of 2-oxoglutarate under the influence of AsAT and its subsequent transformation under the influence of isocitrate dehydrogenase [13]. The activity of AsAT was determined in 0.05 mM potassium phosphate buffer (pH 7.5) containing 0.78 or 1 mM L-glutamate (for cytoplasmic or mitochondrial AsAT, respectively), 4.4 and 3.7 mM oxaloacetate (for cytoplasmic or mitochondrial AsAT, respectively), 0.4 mM NADPH, 0.5 mM $MnCl_2$, 10 mM $NaHCO_3$, and isocitrate dehydrogenase (1 U/ml).

The enzyme quantity converting 1 μmol substrate in 1 min at 25°C was taken as the activity unit (U). The protein content was determined by the Lowry method [14] modified according to [15] for samples containing Triton X-100.

Subcellular location of the enzymes was studied by differential centrifugation [16]. A weighed sample of the rat liver was homogenized in a fourfold volume of isolation medium prepared on the base of 0.05 M Tris-HCl buffer (pH 7.8) (for NADP-IDH) or 0.05 M potassium phosphate buffer (pH 7.8) (for AsAT). The isolation medium contained 0.25 M sucrose, 1 M EDTA, 1% β-mercaptoethanol (for AsAT), and the medium for NADP-IDH was additionally supplemented with 0.5 mM isocitrate. The homogenate was filtered and centrifuged at 5000*g* for 10 min to separate unbroken cellular elements and membranes of organelles. The supernatant was centrifuged again at 18,000*g* for 15 min. The resulting supernatant containing cytoplasmic enzymes was used for a further purification. The precipitate which mainly consisted of mitochondria was washed and then resuspended in the following medium: 0.05 M Tris-HCl buffer (pH 7.8), 1 mM EDTA, 20% glycerol, 1% β-mercaptoethanol, and 0.5 mM isocitrate for NADP-IDH; 0.05 M potassium phosphate buffer (pH 7.8), 1 mM EDTA, 20% glycerol, and 1% β-mercaptoethanol for AsAT. The mitochondria were broken by osmotic shock in a Potter's homogenizer, with 0.1% Triton X-100 for more complete solubilization. The resulting extract was tested for the presence of enzymatic activity. Succinate dehydrogenase (SDH) [17] and lactate dehydrogenase (LDH) [18] were used as mitochondrial and cytoplasmic marker enzymes, respectively.

After the separation of cellular fractions by differential centrifugation, the enzymes were purified further using the following approaches: fractionation with ammonium sulfate (for cytoplasmic NADP-IDH, 35-70% saturation); removal of low-molecular-weight admixtures by gel filtration on Sephadex G-25; ion-exchange chromatography on DEAE-cellulose; gel chromatography on Sephadex G-150. During the gel filtration of NADP-IDH on a column with Sephadex G-25 (1.7 × 20 cm), 0.01 M Tris-HCl buffer (pH 7.8) supplemented with 1.5 mM isocitrate, 2 mM $MnCl_2$, 0.1 mM EDTA, and 0.05 M β-mercaptoethanol was used as an elution medium. The elution medium for AsAT consisted of 0.01 M potassium phosphate buffer (pH 7.8), 1 mM EDTA, and 1% β-mercaptoethanol. During ion-exchange chromatography on DEAE-cellulose, the protein fraction was placed onto a column (1.4 × 13 cm) equilibrated with elution medium of the same composition as in the case with Sephadex G-25. The enzymes were purified using a step-wise gradient of KCl concentration in the eluting buffer. Desorption of NADP-IDH was performed in the gradient of 40-70 mM and that of AsAT in the gradient of 10-35 mM. During the gel chromatography on Sephadex G-150 (2.2 × 65 cm), the elution was performed with the same buffer as for the gel filtration on Sephadex G-25, at the rate of 30 ml/h. All procedures were performed at 0-4°C.

Homogeneity of the enzymes was monitored electrophoretically in 7.5% polyacrylamide gel as described in [19]. The gels were stained with Coomassie Blue R-250 [20]. Molecular weight was determined by gel chromatography on Sephadex G-150 [21]. Inhibition constants were determined as described in [22]. The data were processed by standard statistical methods [23]. The graphs were plotted using data processed with programs of linear and parabolic approximations.

The following reagents and materials were used: Sephadex G-25, Sephadex G-150 (Pharmacia, Sweden); DEAE-cellulose DE-52 (Whatman, Great Britain); Tris

(Serva, Germany); isocitrate, 2-oxoglutarate, oxaloacetate, Triton X-100, malate dehydrogenase (Sigma, USA); NADP, NADPH (Reanal, Hungary); NADH, L-glutamate, L-glutamine, aspartate (ICN, USA). Other reagents were of domestic production, of analytical or chemical purity.

RESULTS

Both NADP-IDH and AsAT were mainly located in the cytoplasm (about 86 and 78% of their total activities in the cell, respectively). The mitochondrial fraction contained about 14% of the NADP-IDH and 22% of the AsAT activities. The distribution of activities of the mitochondrial and cytoplasmic marker enzymes SDH and LDH, respectively, indicated that the majority of cell organelles were not destroyed during the differential centrifugation, but the fractions were cross-contaminated, possibly, because of heterogeneity of the mitochondria. The SDH and LDH activities in the mitochondrial fraction were, respectively, 91 and 9% of their total activities. According to the literature, the distribution of NADP-IDH and AsAT activities in the mitochondrial and cytoplasmic fractions of different animal tissues significantly vary. Thus, the mitochondrial activity of NADP-IDH is 90% of the total activity in the cortex of bovine adrenals, 40% in the cortex of rat kidneys, 30% in rat brain cortex, and 15-20% in the heart and skeletal muscles of rat and in the flight muscle of locust [5]. The mitochondrial activity of AsAT is 21% of the total activity in the human liver [24] and 25-28% in the liver of birds [25]. The ratios of activities of the cytoplasmic and mitochondrial forms of the enzymes seem to be associated with physiological features of different tissues.

Table 1 presents the main parameters of electrophoretically homogenous preparations of mitochondrial and cytoplasmic NADP-IDH, cytoplasmic AsAT, and highly purified mitochondrial AsAT from rat liver purified by the procedures elaborated by us. The specific activity of cytoplasmic NADP-IDH from rat liver prepared by Fatania et al. [9] was 73 U/mg protein, and this value is close to the specific activity of the corresponding enzyme preparation isolated by us. Molecular weights of cytoplasmic and mitochondrial forms of NADP-IDH and AsAT are similar. According to [9], the molecular weight of cytoplasmic NADP-IDH from rat liver is 94 kD, which is slightly lower than the value of 113 kD obtained by us. The molecular weight of NADP-IDH from rat brain is 165 kD [5] and of NADP-IDH from the cytosol of the porcine yellow body is 96 kD [8]. The molecular weight value obtained by us for AsAT is similar to the literature data for the enzyme isolated from other sources. Thus, the molecular weight of AsAT from chicken heart is 92 kD [6], from chicken liver 90 kD [7], and from human liver 95 kD [24]. Optimum pH values for cytoplasmic and mitochondrial NADP-IDH are 7.8 and 7.6, and this is in agreement with the literature data for the enzyme isolated from other sources [4, 8]. The pH optimums for cytoplasmic and mitochondrial forms of AsAT are more different, being 7.5 and 6.8, respectively. The pH optimum for mitochondrial AsAT is similar to values for the enzyme from the human placenta [26], and the pH optimum for cytoplasmic AsAT is close to values for AsAT from the boar spermatozoa [27] and ribbed mussel gill tissue [28].

Isoforms of the enzymes considerably vary in affinities for substrates and cofactors (Table 2), and, obviously, this is important for the regulation of synthesis and utilization of 2-oxoglutarate in different compartments of the cell. In the majority of works generalized values of K_m for different forms of the enzymes are given, and this makes difficult the interpretation of the data [8, 29, 30]. Nevertheless, there are data on K_m for cytoplasmic and mitochondrial NADP-IDH from rat heart [31]. It should be noted that K_m values of differently located forms of the enzyme from rat heart and liver are similar for NADP but slightly differ for isocitrate and Mn^{2+}. This parameter of AsAT significantly varies depending on the source of its isolation and conditions of determination of the activity [26, 32]. However, in many works AsAT is shown to display the greatest affinity for L-aspartate among amino acids and for 2-oxoglutarate among keto acids [29, 30]. The kinetics of the AsAT reaction suggests that every sub-

Table 1. Main parameters of purified cytoplasmic and mitochondrial NADP-IDH and AsAT from rat liver

Form of the enzyme	Specific activity, U/mg protein	Degree of purification	Molecular weight, kD	Electrophoretic mobility, *m*
Cytoplasmic IDH	69.53 ± 3.47	131.2	113 ± 2	0.55
Mitochondrial IDH	66.50 ± 0.82	93.7	96 ± 3	0.36
Cytoplasmic AsAT	48.45 ± 2.39	104.2	98 ± 3	0.22
Mitochondrial AsAT	22.37 ± 0.81	95.2	92 ± 2	—

Note: In Tables 1-5 differences significant at $p < 0.05$ are discussed.

Table 2. Values of K_m of cytoplasmic and mitochondrial NADP-IDH and AsAT from rat liver

Name and form of the enzyme	K_m, mM			
NADP-IDH	isocitrate	Mn^{2+}	Mg^{2+}	NADP
Cytoplasmic form	0.12 ± 0.003	0.43 ± 0.016	0.50 ± 0.013	0.15 ± 0.006
Mitochondrial form	0.07 ± 0.002	0.27 ± 0.012	0.33 ± 0.015	0.06 ± 0.002
AsAT	L-aspartate	2-oxoglutarate	L-glutamate	oxaloacetate
Cytoplasmic form	0.35 ± 0.009	1.25 ± 0.046	0.38 ± 0.012	2.05 ± 0.093
Mitochondrial form	0.75 ± 0.017	1.75 ± 0.057	0.48 ± 0.014	1.84 ± 0.052

Table 3. Substrate inhibition constants of AsAT from rat liver

Form of the enzyme	K_{si}, mM			
	L-aspartate	2-oxoglutarate	L-glutamate	oxaloacetate
Cytoplasmic AsAT	1.20 ± 0.051	3.30 ± 0.163	1.50 ± 0.061	5.70 ± 0.285
Mitochondrial AsAT	1.90 ± 0.085	4.40 ± 0.182	1.20 ± 0.051	4.80 ± 0.205

strate in a high concentration can act as a competitive inhibitor of the enzyme, and this is also confirmed by studies on the enzyme from plant sources [33]. Substrate inhibition constants (K_{si}) obtained by us are presented in Table 3. Unfortunately, there are no data on K_{si} for AsAT in the literature, so it is impossible to compare these parameters.

We were the first to show that some intermediates of the TAC increased the K_m value of AsAT for the substrates but did not change V_{max} of the enzymatic reaction (figure). Thus, in the presence of 0.5 mM isocitrate, which is a substrate of the IDH reaction, the K_m values of both the cytoplasmic and mitochondrial forms of the enzyme increased for L-aspartate. In these isoforms of the enzyme, the effect of succinate on K_m value for L-aspartate was stronger than the effect of isocitrate. Fumarate also increased the K_m value of cytoplasmic AsAT for L-aspartate and 2-oxoglutarate. Citrate increased K_m of the enzyme from the rat cytoplasm for oxaloacetate.

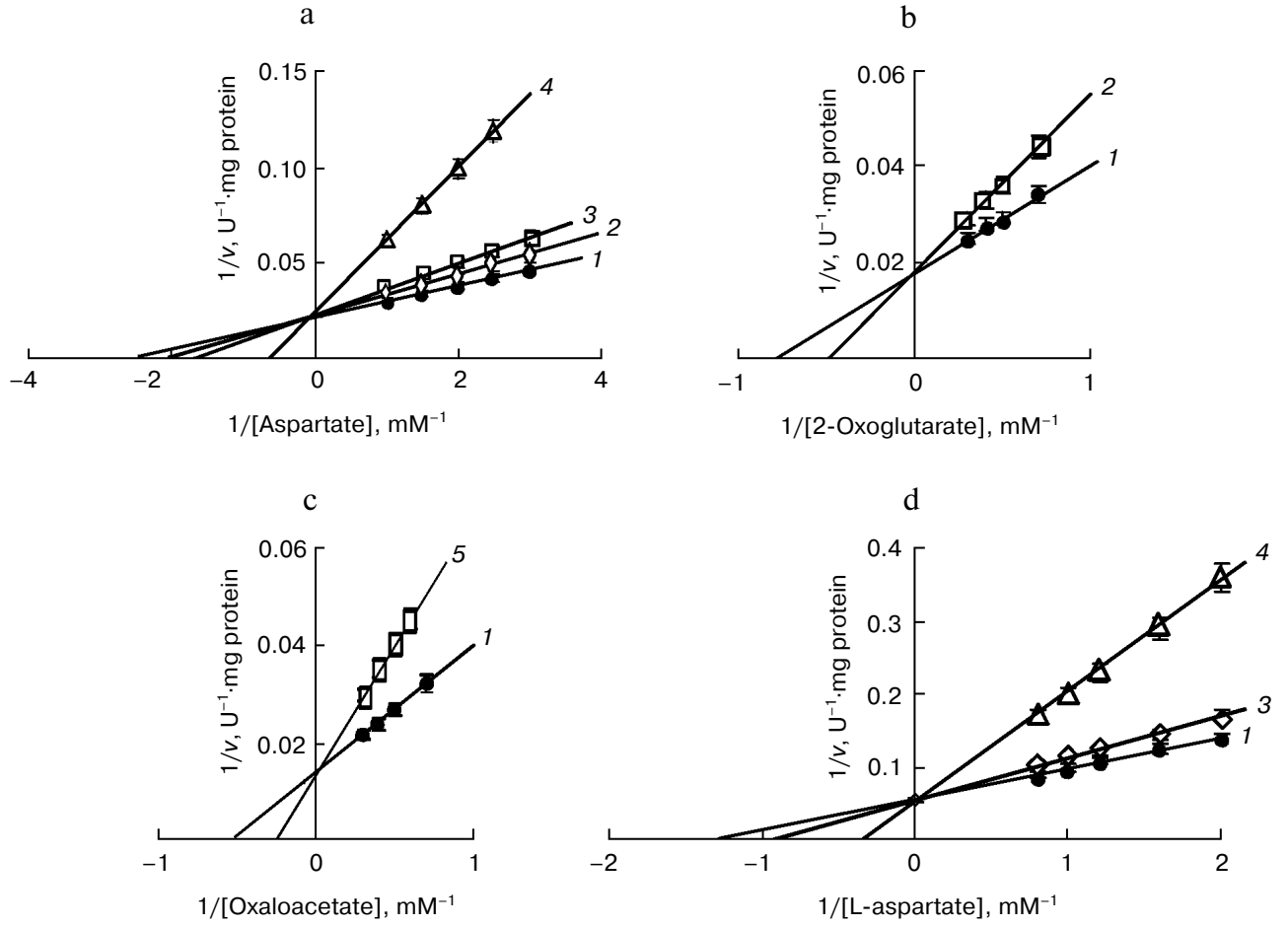
Aspartate, glutamate, and glutamine did not affect the affinity of NADP-IDH for the substrate.

Glutamate, glutamine, and aspartate of key intermediates of nitrogen metabolism were main contributors to the regulation of the NADP-IDH and AsAT activities. During the forward AsAT reaction, glutamate and glutamine competitively inhibited the activity of the mitochondrial isoform more strongly than the activity of the cytoplasmic isoform (Table 4). During the back transamination, aspartate competitively inhibited both isoforms, whereas glutamine was a noncompetitive activator. Glutamate, glutamine, and aspartate influenced the activity of cytoplasmic NADP-IDH that resulted in a non-

Table 4. Constants of inhibition and activation of AsAT and NADP-IDH from rat liver with some amino acids

Metabolite	Constants of inhibition (K_i) and activation (K_a), mM	
	cytoplasmic form	mitochondrial form
	AsAT: forward aminotransferase reaction	
L-Glutamate	K_i = 2.40 ± 0.08	K_i = 1.80 ± 0.07
L-Glutamine	K_i = 3.70 ± 0.14	K_i = 2.60 ± 0.10
	AsAT: back aminotransferase reaction	
L-Aspartate	K_i = 1.60 ± 0.05	K_i = 1.90 ± 0.08
L-Glutamine	K_a = 0.88 ± 0.03	K_a = 0.76 ± 0.02
	NADP-IDH	
L-Glutamate	K_a = 0.28 ± 0.01	no effect
L-Glutamine	K_a = 0.11 ± 0.003	K_i = 0.64 ± 0.02
L-Aspartate	K_a = 1.42 ± 0.07	no effect

competitive and differently pronounced stimulation of the 2-oxoglutarate production during the oxidative decarboxylation of isocitrate. Glutamate and aspartate had no influence on the rate of the IDH reaction catalyzed by mitochondrial NADP-IDH, glutamine at concentrations



Changes in K_m of cytoplasmic (a, b, c) and mitochondrial (d) AsAT from rat liver under the influence of TAC intermediates: the dependence of the reaction rate on the substrate concentration in the absence of TAC metabolites (*1*) and in the presence of fumarate (*2*), isocitrate (*3*), succinate (*4*), and citrate (*5*)

lower than 0.2 mM slightly activated it, and its higher concentrations noncompetitively inhibited the reaction.

2-Oxoglutarate, succinate, oxaloacetate, and *cis*- and *trans*-aconitate competitively inhibited NADP-IDH from the rat liver with respect to isocitrate. Fumarate noncompetitively inhibited the cytoplasmic isoform of the enzyme (Table 5).

The effects of di- and tricarboxylic acids on the activity of AsAT from rat liver were studied, and *cis*- and *trans*-aconitate were shown to noncompetitively activate the cytoplasmic form of the enzyme during the forward transamination (the activation constants are presented in Table 5). Succinate, isocitrate, and fumarate competitively inhibited AsAT with respect to 2-oxoglutarate. The effects of organic acids on the rate of the back transamination catalyzed by AsAT in the rat liver cytoplasm were studied, and succinate, malate, and *trans*-aconitate were found to be competitive inhibitors with respect to the keto-substrate. Citrate stimulated the catalytic activity of AsAT. *cis*-Aconitate and fumarate did not influence the rate of 2-ketoglutarate production during the back transamination. In rat liver mitochondria, succinate and malate influenced the rate of metabolic transformations of 2-oxoglutarate catalyzed by AsAT.

DISCUSSION

The presence of two main pools (cytoplasmic and mitochondrial) of the NADP-IDH and AsAT activities in rat liver suggests the coupling and co-regulation of reactions of carbon and nitrogen metabolism in these compartments of the cell. Because the activities of the enzymes are mainly displayed in the cytoplasm, the reactions catalyzed by cytoplasmic NADP-IDH and AsAT seem to be main loci of metabolic transformations of 2-oxoglutarate, which serves a source of carbon skeletons in the biosynthesis of glutamate and other amino acids. Consequently, the reactions catalyzed by the cytoplasmic forms of the enzymes can be the most important points of regulation and coordination of carbon and nitrogen metabolism in the cell.

Table 5. Inhibition and activation constants of AsAT and NADP-IDH from rat liver with some organic acids

Metabolite	Constants of inhibition (K_i) and activation (K_a), mM	
	cytoplasmic form	mitochondrial form
	NADP-IDH	
2-Oxoglutarate	$K_i = 0.25 \pm 0.009$	$K_i = 0.38 \pm 0.011$
Succinate	$K_i = 0.48 \pm 0.012$	$K_i = 0.56 \pm 0.025$
Oxaloacetate	$K_i = 1.72 \pm 0.055$	$K_i = 1.50 \pm 0.070$
Citrate	no effect	$K_i = 1.40 \pm 0.055$
cis-Aconitate	$K_i = 1.80 \pm 0.067$	$K_i = 1.63 \pm 0.071$
trans-Aconitate	$K_i = 2.20 \pm 0.095$	$K_i = 1.90 \pm 0.085$
Fumarate	$K_i = 2.32 \pm 0.101$	no effect
	AsAT: forward aminotransferase reaction	
cis-Aconitate	$K_a = 0.42 \pm 0.020$	no effect
trans-Aconitate	$K_a = 0.49 \pm 0.020$	no effect
Isocitrate	$K_i = 6.60 \pm 0.141$	no effect
Succinate	$K_i = 5.70 \pm 0.122$	$K_i = 4.10 \pm 0.150$
Fumarate	$K_i = 8.40 \pm 0.200$	no effect
	AsAT: back aminotransferase reaction	
trans-Aconitate	$K_i = 11.30 \pm 0.231$	no effect
Succinate	$K_i = 7.80 \pm 0.191$	$K_i = 6.30 \pm 0.130$
Malate	$K_i = 9.50 \pm 0.210$	$K_i = 8.70 \pm 0.170$

Along with similarity in molecular weights and pH optimums of cytoplasmic and mitochondrial NADP-IDH and AsAT, these isoforms of the enzymes are characterized by some specific features of the catalytic effects. Thus, mitochondrial and cytoplasmic NADP-IDH from rat liver have different values of K_m. Analysis of kinetic parameters of AsAT shows that the equilibrium of transamination in the cytoplasm is displaced towards the generation of L-glutamate and oxaloacetate. Obviously, the necessary substrate for the AsAT reaction in the cytoplasm, 2-oxoglutarate, is mainly produced during the reaction catalyzed by cytoplasmic NADP-IDH. It seems that both the mitochondrial and cytoplasmic pools of 2-oxoglutarate can be replenished at the expense of activity of mitochondrial AsAT because the equilibrium of transamination in mitochondria is displaced towards the production of this metabolite. This hypothesis is supported by the lower chemical stability of oxaloacetate as compared to 2-oxoglutarate [33], and this seems to be favorable for production of L-aspartate and 2-oxoglutarate. And it should be emphasized that 2-oxoglutarate generated during the IDH reaction in the mitochondria is mainly transformed in the TAC under the influence of the ketoglutarate dehydrogenase complex, and only its small fraction can be transmitted into the cytoplasm [34]. The inability of direct entering of oxaloacetate from mitochondria into the cytoplasm is known. Catalyzed by mitochondrial AsAT, oxaloacetate is changed to 2-oxoglutarate, which is afterwards transferred into the cytoplasm where, under the influence of the cytoplasmic form of the enzyme, can react with L-aspartate and produce oxaloacetate and L-glutamate. Thus, the different sensitivity of the enzymes to concentrations of the substrates and cofactors can be important for integrating processes of 2-oxoglutarate metabolism occurring in different compartments of the cell.

The kinetics of the AsAT reaction suggests that each substrate in excess concentration can act as a competitive inhibitor of the enzyme. Different sensitivity of the enzyme isoforms to inhibitory effects of the substrates characterized by different values of K_{si} is likely to be important for displacement of the transamination equilibrium to the generation or utilization of 2-oxoglutarate in different cellular compartments. The transamination equilibrium can be markedly influenced by structural analogs of the substrates, i.e., some intermediates of the TAC and some amino acids. Thus, glutamate and glutamine slow down the rate of the forward aspartate aminotransferase reaction, whereas aspartate inhibits and glutamine activates the back transamination. Some TAC metabolites (including isocitrate, which is a substrate of the IDH reaction) increase the K_m of AsAT for the substrates.

Some amino acids studied (glutamate, glutamine, and aspartate) did not change the K_m values for the substrate of NADP-IDH but increased V_{max}. The rate of synthesis of 2-oxoglutarate, which is a metabolic precursor of glutamate and glutamine, most considerably increased during the IDH reaction at low concentrations of these amino acids. With an increase in the concentrations of these metabolites in the cell the activity of NADP-IDH was inhibited. It seemed to be associated with the displacement of the AsAT reaction equilibrium towards the back transamination. On the contrary, low concentrations of aspartate had virtually no effect on the IDH reaction, while its higher concentrations stimulated the catalytic effect of NADP-IDH. This seemed to be associated with the displacement of the AsAT reaction towards the forward transamination resulting in the utilization of 2-oxoglutarate and increase in the rate of oxidative decarboxylation of isocitrate because 2-oxoglutarate was a strong competitive inhibitor of NADP-IDH. The competitive inhibition by 2-oxoglutarate has been also shown for NADP-IDH from some other sources [4, 5, 34]. Obviously, the inhibition with 2-oxoglutarate occurs on the feedback principle and is a universal mechanism for regulation of the enzyme activity in various organisms [5]. Another substrate of the AsAT-reaction, oxaloacetate,

was also a competitive inhibitor of NADP-IDH. But its inhibitory effect was manifested at higher concentrations than a similar effect of 2-oxoglutarate. Possibly, an increase in the oxaloacetate concentration in the cell was associated with an acceleration of synthesis of 2-oxoglutarate at the expense of the back AsAT reaction and inhibition of its generation during the IDH reaction.

Thus, the regulation of AsAT and NADP-IDH in the cell is multiple and often oppositely directed. The findings suggest a possible coordination between functioning of AsAT and NADP-IDH in the presence of some metabolites. Differently located forms of the enzymes are likely to influence the carbon and nitrogen metabolism via the distribution of 2-oxoglutarate flows between both different cell compartments and metabolic pathways in them. Changes in the enzyme activities under the influence of crucial intermediates of the TAC and nitrogen metabolism are likely to be the most important mechanisms of the regulation.

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