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Induction of Aconitate Hydratase in Hepatocytes of Starving Rats

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Abstract—Induction of the activity of aconitate hydratase (AH) was observed in rat hepatocytes under the conditions of food deprivation. The increase in AH activity after 4 days of starvation in the studied tissues was from 0.57 to 2.05 U/g crude liver weight. The induction of aconitase was associated both with the cytoplasmic and mitochondrial AH isoforms. The activities of cytosolic and mitochondrial AH isoforms in starving animals consisted of 83 and 17% of the total activity, respectively. The cytoplasmic and mitochondrial isoforms of the enzyme with specific activities 11.1 and 6.13 U/mg protein, respectively, were obtained by a five-step purification procedure that included fractionation with ammonium sulfate, ion-exchanging chromatography on DEAE-Toyopearl and gel filtration. The purified preparations of these AH isoforms were electrophoretically homogenous. The molecular weights of these isoforms were estimated and several kinetic and regulatory properties were studied.

Key words: aconitate hydratase, food deprivation, hepatocytes, glyoxylate cycle, citric acid cycle, gluconeogenesis, purification, kinetic characteristics

Extreme environmental conditions influence an organism, often leading to significant changes in its physiological and metabolic functions and the appearance of stress. Starvation is one of the stress-factors, which significantly alters the energetic status of the cell. The decrease in nutrient delivery to the organism leads to the mobilization of nutrient reserves. Under these circumstances the cooperation between organs plays an important role in the resolution of the energetic problems on the level of the whole organism. The liver plays a particular role in the cooperation between systems: gluconeogenetic processes occur in the liver leading to glucose synthesis; ketone bodies, an active transport form of fat, are also synthesized in the liver. Both glucose and ketone bodies are the most important energy substrates of the organism that are utilized by other organs [1].

The mobilization of all resources of the organism directed towards the maintenance of energy balance of the cells was demonstrated during the study of food deprivation. First of all the most available resources are consumed, in particular that of glucose and then of glycogen. It was shown that glycogen content in hepatocytes decreased significantly already at the end of the first 24 h of starvation [1, 2], and the switch to the metabolism of fatty acids occurred. Such amino acids as aspartate and glutamate are also used as energy substrates during starvation since their transamination leads to the formation of substrates for the citric acid cycle.

The citric acid cycle plays the key role both in the mobilization of the reserved nutrients and in ensuring gluconeogenesis from fatty acids that is observed on the 3-4th day of starvation. This process apparently maintains stable glucose concentration in blood during its limited entrance from outside and may ensure the phenomenon of glycogen resynthesis on the 4th day of food deprivation that was observed earlier [2]. We demonstrated earlier that the biochemical mechanism of carbohydrate synthesis from lipid reserves during starvation represented the induction of the enzymes of the glyoxylate cycle, which condensed two molecules of acetyl-CoA that were formed during β-oxidation of fatty acids into a glucogenic substrate—succinate [3].

Aconitate hydratase (aconitase, AH, EC 4.2.1.3) is one of the enzymes that are involved in the functioning of both the citric acid cycle and the glyoxylate cycle. It catalyzes the reactions of mutual conversion of citrate, *cis*-aconitate, and isocitrate [4]. AH is found in animal tissues as two isoenzymes with cytoplasmic and mitochondrial localization. A third gene that codes for the glyoxysomal form of the enzyme was identified in plants [5, 6].

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AH activity has been detected in almost all living organisms [7, 8]. In particular, it has been found in lung, heart, liver, and skeletal muscle of rat [9-12]. AH was isolated and studied from pig heart [13] and several other sources [4]. Mitochondrial and cytoplasmic AH forms were also obtained from rat liver [10, 14]. It was shown that mitochondrial AH differs from cytoplasmic AH in a number of kinetic parameters and regulatory properties in plants, microorganisms, and animals [10, 15, 16].

The aims of this work were to investigate the possibility of induction of the cytoplasmic and mitochondrial AH isoforms in hepatocytes under conditions of food deprivation. The AH isoforms were isolated and their physicochemical, kinetic characteristics, and regulatory mechanisms were studied.

MATERIALS AND METHODS

Laboratory rats (*Rattus rattus* L.) fed normally during growth at the age of three months weighting 250-300 g were the objects of the study. The animals were subjected to food deprivation with unlimited access to water for periods up to six days. Animals receiving the normal ration were used as controls.

Before liver extraction, the animals were anesthetized with diethyl ether and decapitated. The subcellular fractions were isolated by homogenization of 1 g of liver in 5 ml of the medium for isolation containing 50 mM Tris-HCl, pH 8.0, 0.3 M sucrose, 5 mM sodium citrate, 1 mM $MgCl_2$, 4 mM dithiothreitol (DTT), and 1 mM EDTA. The non-dissociated tissues were pelleted by centrifugation at 3000*g* for 5 min. For isolation of the mitochondrial fraction the supernatant was centrifuged at 15,000*g* for 15 min and washed with the isolation medium. The pellet resulting from the second centrifugation (15,000*g* for 15 min) was carefully resuspended in the homogenization medium (without sucrose) with addition of 1% Tween-80.

The AH activity was measured spectrophotometrically at 240 nm by differences in the absorption of citrate and isocitrate in comparison to *cis*-aconitic acid. The reaction mixture contained 50 mM Tris-HCl, pH 8.0, 2 mM sodium isocitrate, 1 mM $MgCl_2$, and 1 mM DTT. The quantity of the enzyme that converts 1 μmol of the substrate during 1 min at 25°C at optimal pH was taken as 1 U of the enzymatic activity. Protein content was measured according to the method of Lowry et al. [17].

The enzyme was purified by the following steps: 1) fractionation by ammonium sulfate (35-65% of saturation); 2) desalting of the resulting protein preparation by gel filtration. A column with Sephadex G-25 (1.7 × 15 cm) (Pharmacia, Sweden) was equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 1 mM DTT and 1 mM $MgCl_2$; 3) ion-exchange chromatography on DEAE-Toyopearl. The protein fraction was applied on the column (1.5 × 13 cm) equilibrated with 10 mM Tris-HCl buffer, pH 8.0. The protein was eluted by a step gradient of KCl; 4) gel filtration on a column with Toyopearl HW-65 (Toyo Soda, Japan) (2.0 × 55 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl, 1 mM DTT, 1 mM EDTA, and 1 mM $MgCl_2$. All steps were carried out in a cold room at 0-4°C.

Electrophoresis was carried out in 7.5% polyacrylamide gel according to a modification of the method of Davis. Proteins were universally stained with amido black 10B. For the specific identification of AH we used developing medium of the following content: 50 mM Tris-HCl, pH 8.0, 20 mM *cis*-aconitic acid, 2 mM $MgCl_2$, 1 unit of $NADP^+$-dependent isocitrate dehydrogenase, 1 mM $NADP^+$, 3.3 mM phenazine methosulfate (PMS), nitro blue tetrazolium (0.5 mg/ml). The molecular weight was estimated by electrophoresis (PAGE) in the presence of SDS using the following marker proteins: BSA (bovine serum albumin) (66 kD), ovalbumin (43 kD), carboanhydrase (31 kD), trypsin (21 kD), and lysozyme (14 kD).

The molecular weight of the enzyme was measured by gel chromatography on Toyopearl HW-65 using the calibration curve that was establish using marker proteins: catalase (120 kD), BSA (66 kD), egg albumin (44 kD), peroxidase (45 kD), and cytochrome *c* (12.5 kD).

The data presented in tables and figures are the mean values ± standard deviation. The number of independent experiments is presented in parentheses.

RESULTS

Experimental animals that were subjected to the food deprivation demonstrated a more than 3-fold increase in AH activity in the studied tissue. This value was maximal at the 4th day of starvation and equaled 2.05 U/g crude weight of the homogenate of liver tissues. The activity decreased on the 6th day and the experiment was terminated (Fig. 1).

We observed the induction of both cytoplasmic and mitochondrial AH isoforms in hepatocytes of the control and experimental rats. It was shown that in both cases the maximal enzymatic activity was detected in the cytoplasmic fraction. According to the results the relative values of the activities of cytosolic and mitochondrial forms of aconitase were 79 and 21% (0.80 and 0.18 U) for control animals and 83 and 17% (2.00 and 0.53 U) for starving animals.

To study the physical, chemical, and regulatory properties of these AH isoforms from cytoplasm and mitochondria of rat hepatocytes under conditions of food deprivation, they were purified. Typical results are presented in Tables 1 and 2.

The successive use of fractionation with ammonium sulfate, gel filtration on Sephadex G-25, ion-exchange chromatography on DEAE-Toyopearl, and gel chro-

matography on Toyopearl HW-65 produced the preparation of the cytoplasmic enzyme isoform from hepatocytes of starved rats with specific activity 11.1 U/mg corresponding to 232-fold purification and 10% yield, and the preparation of the mitochondrial isoform with specific activity 6.13 U/mg corresponding to 123-fold purification and 15% yield.

During the experiments we elaborated conditions for long-term storage of AH isoforms. The activity of highly purified cytoplasmic aconitase decreased by 15-20% during the first 24 h of storage at 4°C and then by ~5% every 24 h. During the study of the mitochondrial enzyme it was shown that under the same conditions its activity decreased much more rapidly in comparison to the cytoplasmic enzyme: by 35-40% during the first 24 h and then by ~15% every 24 h. The optimal conditions for AH were as follows: medium containing 0.1 M Tris-HCl, pH 7.8-8.0, 2 mM β-mercaptoethanol or 8 mM DTT, 4 mM $MgCl_2$, 25% glycerol, and temperature 15°C in both cases. Under these conditions during the first five days the cytoplasmic and mitochondrial enzymes retained 90 and 70% of the initial activity, respectively, and after one month, 50 and 30%, respectively.

The electrophoretic study of the resulting preparations of AH isoforms in polyacrylamide gel under nondenaturing conditions demonstrated the presence of a single protein band with R_f equal to 0.7 for the cytoplasmic isoform and 0.62 for the mitochondrial isoform. Using the development specific for the activity we showed that the resulting homogenous protein preparations corresponded to aconitase (Fig. 2).

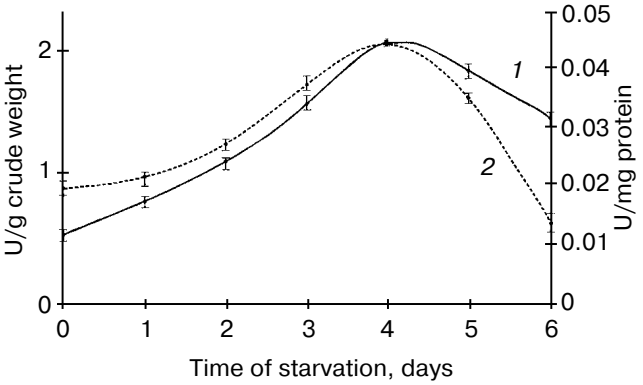

Fig. 1. Change in the aconitase activity in rat hepatocytes during starvation: *1*) U/g crude weight; *2*) U/mg protein (n = 10).

The molecular weight values obtained by gel filtration on Sephadex G-150 and Toyopearl HW-65 were 96

Table 1. Purification of cytoplasmic aconitate hydratase from hepatocytes of starving rats

Purification step	Volume, ml	Total activity, U	Protein quantity, mg	Specific activity, U/mg protein	Yield, %	Purification degree
Cytosolic fraction	30.5	11.5	238.2	0.048	100	1
Fractionation with $(NH_4)_2SO_4$, 35-65%	5.0	8.5	82.5	0.103	74	2.2
Gel filtration on Sephadex G-25	8.0	6.1	31.0	0.197	53	4.1
Chromatography on DEAE-Toyopearl	9.5	2.9	0.72	4.03	25	84
Gel filtration on Toyopearl HW-65	6.0	1.1	0.099	11.1	10	232

Table 2. Purification of mitochondrial aconitate hydratase from hepatocytes of starving rats

Purification step	Volume, ml	Total activity, U	Protein quantity, mg	Specific activity, U/mg protein	Yield, %	Purification degree
Mitochondrial fraction	2.0	2.60	51.2	0.050	100	1
Fractionation with $(NH_4)_2SO_4$, 35-65%	4.8	2.10	20.0	0.105	81	2.1
Gel filtration on Sephadex G-25	10.0	1.05	6.3	0.167	40	3.3
Chromatography on DEAE-Toyopearl	7.5	0.64	0.307	2.09	25	41.7
Gel filtration on Toyopearl HW-65	5.0	0.38	0.062	6.13	15	123

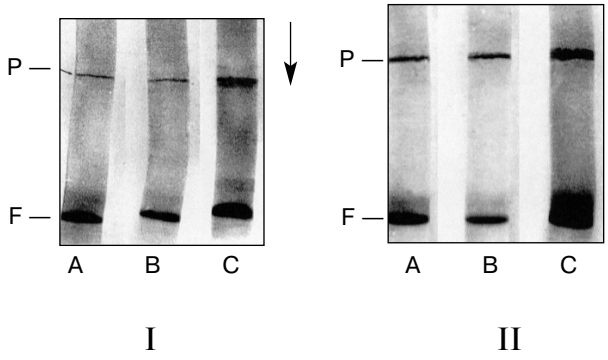

Fig. 2. Electrophoregrams of aconitate hydratase from rat hepatocytes: I) cytoplasmic AH; II) mitochondrial AH; A, B) universal staining with amido black 10B; C) specific staining; P) protein band; F) front of bromophenol blue; the arrow indicates the direction of protein migration during electrophoresis.

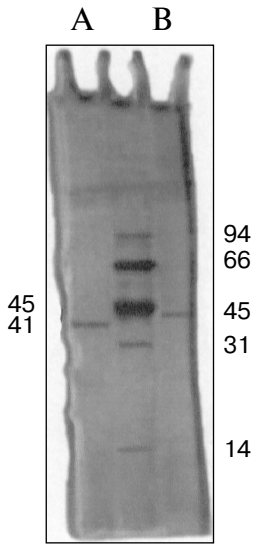

Fig. 3. Assessment of the aconitate hydratase molecular weight by SDS-PAGE. On the right: cellulase, BSA, ovalbumin, carboanhydrase, lysozyme; on the left: cytoplasmic (B) and mitochondrial (A) AH isoforms.

and 84 kD for the cytoplasmic and mitochondrial aconitase isoforms, respectively. Both enzymes are homodimers: the electrophoresis in the presence of SDS showed that the molecular weights of the subunits of the mitochondrial and the cytoplasmic AH were 45 and 41 kD, respectively (Fig. 3). It was demonstrated earlier that AH from pig heart is a dimeric enzyme with molecular weight of 89 kD [18]. Both monomeric and dimeric AH forms have been found in plants with molecular weights 104.5 kD for pea, 106 kD for sunflower, and 102 kD for maize [15]. The molecular weights of the two isoenzymes from *Bacillus cereus* were 160 and 80 kD. The mitochondrial AH isoform from *E. coli* has molecular weight of about 83 kD [16].

The study of the kinetic and regulatory properties of AH showed that the enzyme functions according to Michaelis–Menten kinetics. The K_m values for citrate, isocitrate, and *cis*-aconitate for the cytoplasmic and mitochondrial AH isoforms from hepatocytes of starving rats are presented in Table 3. The catalytic constant (k_{cat}) for the mitochondrial AH isoform was 4.5-fold smaller than for the cytosolic enzyme. Analysis of the results showed that the K_m value was minimal for *cis*-aconitate. The following buffered systems were used for the determination of the pH optimum: Tris-HCl, NaH_2PO_4-Na_2HPO_4. AH had the maximal activity in the pH interval 6.8-8.0 (Table 3); for the cytosolic AH form the pH optimum was shifted towards basic pH values in comparison to the mitochondrial isoform.

The study of the influence of intermediates of cellular metabolism on the activity of the cytoplasmic and mitochondrial AH isoforms from rat hepatocytes revealed inhibition by malate, succinate, glyoxylate, oxaloacetate, and fumarate. The K_i values (with isocitrate as substrate) calculated according to the method of Dixon [19] are presented in Table 4. The K_i values for glucose-1-phos-

Table 3. Several catalytic properties of aconitate hydratase isoforms from hepatocytes of starving rats ($n = 6$)

Enzyme isoform	K_m, μM			k_{cat}, U/mg	pH optimum	
	citrate	isocitrate	*cis*-aconitate	citrate	Tris-HCl buffer	sodium phosphate buffer
Cytoplasmic	93.7 ± 2.0	34.1 ± 0.2	14.7 ± 0.4	2.06 ± 0.02	8.0 ± 0.1	7.2 ± 0.2
Mitochondrial	24.3 ± 1.4	11.4 ± 0.2	4.2 ± 0.1	0.45 ± 0.01	7.4 ± 0.1	6.8 ± 0.1

phate and glucose-6-phosphate were 1.9 and 2.35 mM for the cytoplasmic and 0.81 and 1.14 mM for the mitochondrial isoform (Fig. 4). The inhibitory effect on AH activity was not detected in all experiments when malonate, 2-oxyglutarate, and glycolate were used. The incubation of the isoenzymes with such amino acids as serine, glycine, and aspartic and glutamic acids did not induce any significant change in the enzymatic activity.

Study of the influence of hydrogen peroxide on the isoforms of the animal aconitase showed 90-95% inhibition with peroxide concentration 2.3 mM for the cytoplasmic and 1.5 mM for the mitochondrial AH isoforms (Fig. 5). The possible mechanism of inhibition could be the presence in the active center of AH isoforms of Fe-S clusters that are destroyed in the presence of H_2O_2 [20, 21].

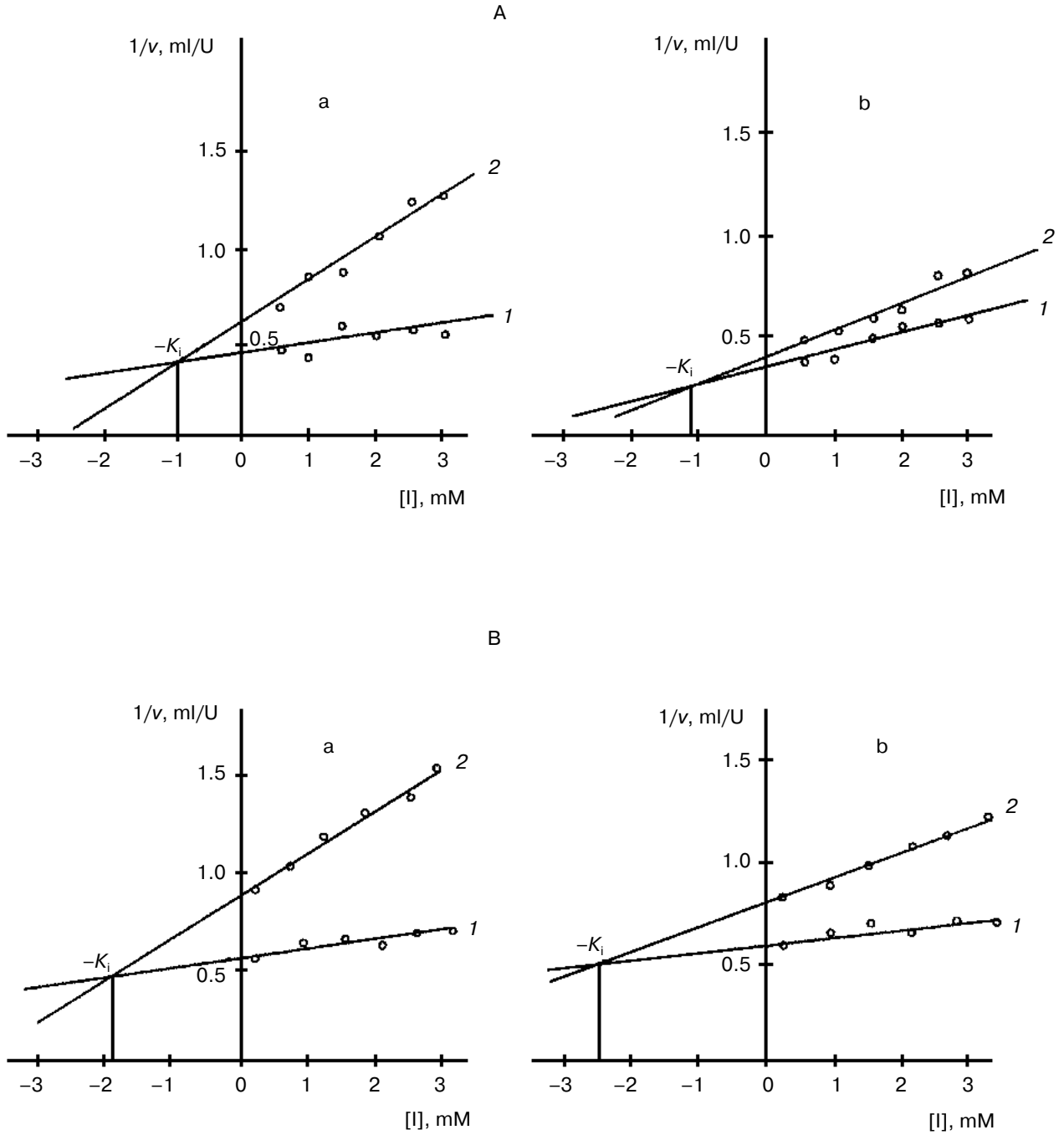

Fig. 4. Measurement of the inhibition constant of cytoplasmic (A) and mitochondrial (B) forms of aconitate hydratase from rat hepatocytes for glucose-1-phosphate (a) and glucose-6-phosphate (b). Isocitrate concentration (mM): *1*) 4; *2*) 2.

Table 4. Values of inhibition constants (K_i, mM) of aconitate hydratase activity (from rat hepatocytes) by the intermediates of cellular metabolism ($n = 6$)

Metabolite	Enzyme isoform	
	cytoplasmic	mitochondrial
Trans-aconitate	3.41 ± 0.15	1.37 ± 0.05
Succinate	0.61 ± 0.01	0.33 ± 0.01
Glyoxylate	0.42 ± 0.01	0.14 ± 0.003
Oxaloacetate	2.80 ± 0.07	1.03 ± 0.02
Malate	1.81 ± 0.03	2.94 ± 0.07
Fumarate	0.51 ± 0.01	0.40 ± 0.01
Glucose-1-phosphate	1.90 ± 0.03	2.35 ± 0.06
Glucose-6-phosphate	0.81 ± 0.01	1.14 ± 0.02

The study of the influence of Fe^{2+} demonstrated that at $FeCl_2$ concentration 0.1-1 mM and cysteine 0.05-0.5 mM both enzyme forms were activated (Fig. 6). The indicated concentrations of Fe^{2+} and cysteine were chosen because these substances activated AH from other sources in these concentration ranges [22, 23]. The AH activity increased 2-4-fold in the presence of both Fe^{2+} and cysteine.

DISCUSSION

New metabolic demands arise for an organism under stress conditions. Quantitative and qualitative changes in the cellular enzymatic systems could be necessary for their satisfaction depending on the strength and type of the external effects. Adaptive changes include alteration of the regulatory mechanisms of the metabolic pathways leading to the variation of the isoenzymatic spectrum or of the catalytic properties of the existing enzymes [24].

The increase in the activities of the cytoplasmic and mitochondrial AH isoforms during starvation shown in this study provides evidence for the acceleration of metabolic processes that are linked to this enzyme. The mitochondrial AH form mainly supports the functioning of the citric acid cycle. Activation of the mitochondrial oxidative processes apparently plays the key role in providing energy for the cell under stress conditions when an organism consumes more energy for biosynthesis and maintaining of homeostasis [24].

The cytoplasmic AH form may play two roles. First, the conversion of citrate into isocitrate provides the substrate for NADP-dependent isocitrate dehydrogenase and the following formation of glutamate [4]. Second, the induction of the cytosolic aconitase may ensure the induction of the enzymes of the glyoxylate cycle in hepatocytes during food deprivation. We have observed earlier the appearance of the activities of isocitrate lyase and malate synthase together with the increase in the activity of the peroxysomal malate dehydrogenase in rat hepatocytes during food deprivation and experimentally induced

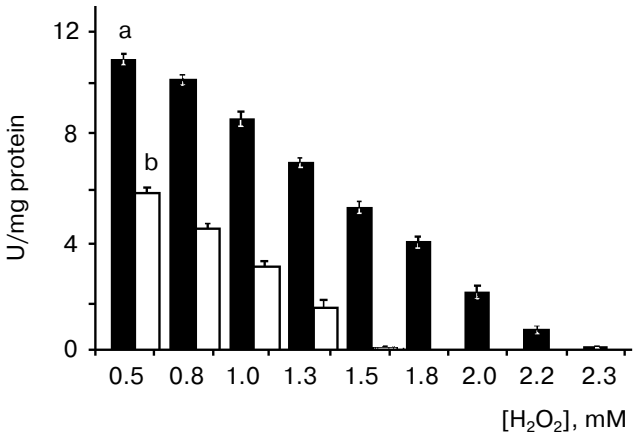

Fig. 5. Dependence of the activity of cytoplasmic (a) and mitochondrial (b) isoforms of aconitate hydratase from rat hepatocytes on hydrogen peroxide concentration (isocitrate concentration, 2 mM) ($n = 6$).

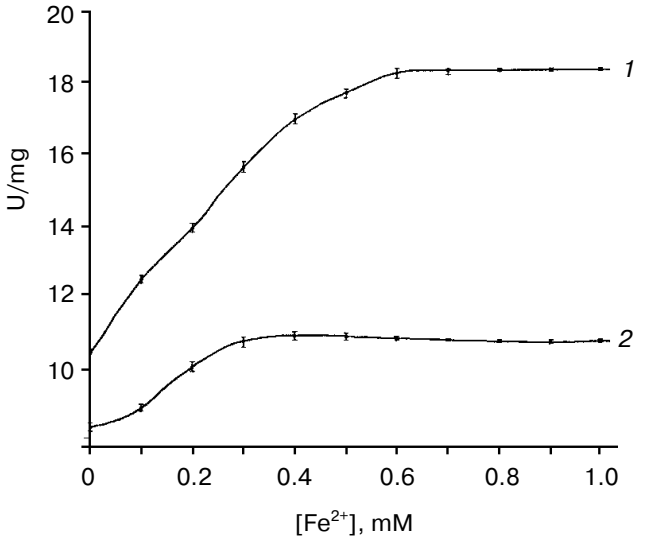

Fig. 6. Influence of Fe^{2+} and cysteine on the activity of cytoplasmic (*1*) and mitochondrial (*2*) isoforms of aconitate hydratase from the liver of starving rats (isocitrate concentration, 2 mM; cysteine concentration, 0.1 mM + ([Fe^{2+}]/2.2) mM ($n = 6$).

diabetes [3, 25, 26]. The increase in the activities of aconitase (demonstrated in this study) and citrate synthase is also necessary for the full functioning of the glyoxylate cycle.

The isolation of the homogenous mitochondrial and cytoplasmic forms of the enzyme allowed the study of their catalytic and regulatory properties and their comparison with the properties of the enzymes from the other sources [4]. The resulting preparations of aconitase were similar to the enzyme isolated earlier from tissues of normal animals in the specific activity of the electrophoretically homogenous preparations, the values of Michaelis constants, and pH optima of the activities [10, 14]. These data demonstrate that the change in the total AH activity during starvation was due mainly to the increase in the enzyme quantity.

It was shown that aconitase from hepatocytes of starving rats can be inhibited by organic acids like malate, succinate, glyoxylate, oxaloacetate, *trans*-aconitate, fumarate, the resistance to these intermediates being significantly higher for the cytosolic form of the enzyme. Glyoxylate, succinate, and fumarate are the most efficient inhibitors of the mitochondrial AH. The accumulation of the two last intermediates of the citric acid cycle may be due to the inhibition of the succinate dehydrogenase and fumarate hydratase, or to the activation of the dicarboxylate carrier that transports succinate. The latter is synthesized in peroxysomes in the glyoxylate cycle [3]. In this case the inhibition of aconitase allows the utilization of dicarboxylic acids for the gluconeogenetic processes. The cytosolic enzyme form was also more resistant during storage and against hydrogen peroxide that destroyed the Fe-S clusters of the enzyme.

Thus, we demonstrated in this study the induction of both cytoplasmic and mitochondrial AH isoforms in hepatocytes during food deprivation. These isoforms were isolated and their kinetic properties and regulatory pathways were determined.

REFERENCES

1. Lebkova, N. P. (2000) *Vestnik RAMN*, **9**, 3-12.
2. Lebkova, N. P., Bondarenko, M. F., and Kolesnikova, O. E. (1980) *Byul. Eksp. Biol. Med.*, **5**, 614-617.
3. Popov, V. N., Igamberdiev, A. U., Schnarenberger, C., and Volvenkin, S. V. (1996) *FEBS Lett.*, **390**, 258-260.
4. Glusker, J. P. (1971) *Enzymes*, **5**, 413-439.
5. Slaughter, C. A., Hopkinson, D. A., and Harris, H. (1975) *Ann. Hum. Genet.*, **39**, 193-202.
6. Prodromou, C., Artymiuk, P. J., and Guest, J. R. (1992) *Eur. J. Biochem.*, **204**, 599-609.
7. Scholze, H. (1983) *Biochim. Biophys. Acta*, **763**, 133-137.
8. Eprintsev, A. T., Zemlyanukhin, L. A., and Aleksyuk, M. P. (1995) *Biochemistry (Moscow)*, **80**, 939-944.
9. Evelson, P., and Gonzalez-Flecha, B. (2000) *Biochim. Biophys. Acta*, **1523**, 209-216.
10. Konstantinova, S. G., and Russanov, E. M. (1996) *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.*, **113**, 125-130.
11. Konstantinova, S. G., Jordanova, N. G., and Russanov, E. M. (2000) *Acta. Physiol. Pharmacol. Bulg.*, **25**, 33-42.
12. Andersson, U., Leighton, B., Young, M. E., Blomstrand, E., and Newsholme, E. A. (1998) *Biochem. Biophys. Res. Commun.*, **249**, 512-516.
13. Villafranca, J. (1974) *J. Biol. Chem.*, **249**, 6149-6155.
14. Guarriera-Bobyleva, V., Volpi-Bobochi, M. A., and Masini, A. (1973) *Eur. J. Biochem.*, **34**, 455-458.
15. Zemlyanukhin, A. A., Eprintsev, A. T., Zemlyanukhin, L. A., and Igamberdiev, A. U. (1984) *Fiziol. Rast.*, **31**, 337-343.
16. Agrawal, P., Garg, G., and Gollakota, K. (1976) *Biochem. Biophys. Res. Commun.*, **70**, 987-996.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
18. Villafranca, J., and Mildvan, A. (1971) *J. Biol. Chem.*, **246**, 5791-5798.
19. Dixon, M., and Webb, E. (1982) *Enzymes* [Russian translation], Nauka, Moscow.
20. Eisenstein, R. S., Barton, H. A., Pettingell, W. H., Jr., and Bomford, A. B. (1997) *Arch. Biochem. Biophys.*, **343**, 81-91.
21. Emptage, M. H., Dreyer, J. L., Kennedy, M. C., and Beinert, H. (1993) *J. Biol. Chem.*, **219**, 11106-11111.
22. Beinert, H., Kennedy, M. C., and Stout, C. D. (1996) *Chem. Rev.*, **96**, 2335-2373.
23. Philpott, C. C., Klausner, R. D., and Rouault, T. A. (1994) *Proc. Natl. Acad. Sci. USA*, **9**, 7321-7325.
24. Hochachka, P., and Somero, J. (1988) *Biochemical Adaptation* [Russian translation], Mir, Moscow.
25. Popov, V. N., Volvenkin, S. V., Eprintsev, A. T., and Igamberdiev, A. U. (1998) *FEBS Lett.*, **440**, 55-58.
26. Popov, V. N., Volvenkin, S. V., Eprintsev, A. T., and Igamberdiev, A. U. (2000) *Izv. RAN. Ser. Biol.*, **6**, 672-678.