ROLE OF AMINOACYL-*t*RNA-SYNTHETASES IN CHANGES IN RATE OF PROTEIN BIOSYNTHESIS IN RABBIT LIVER DURING MYOCARDIAL ISCHEMIA

Z. P. Martinkus, L. L. Ivanov, A. V. Liekis, and A. K. Praškevičius

UDC 616.127-005.4-092.9-07:616.36-008.939.6

KEY WORDS: experimental myocardial ischemia; aminoacyl-*t*RNA-synthetase, cell-free protein-synthesizing system of the liver.

An extremely important function in protein biosynthesis is performed by the aminoacyl-*t*RNA-synthetases (ARSases), which catalyze strictly specific aminoacylation of *t*RNA [3]. A characteristic property of the ARSases of multicellular organisms is their ability to function in the composition of macromolecular complexes [3, 6]. It has been shown, moreover, that when certain physiological and pathological changes take place in the organism, leading to disturbance of protein biosynthesis, there is a redistribution of ARSase activity between complexes of these enzymes of different molecular weight or between complexes and free enzymes [7-9].

We showed previously that protein biosynthesis is disturbed in the liver of rabbits with experimental myocardial ischemia (EMI) [4] and activity of components of the translation apparatus is modified [2, 5]. In the investigation described below the composition of macromolecular complexes of ARSases from rabbit liver was studied and their activity toward the rate of protein synthesis in a cell-free system was determined under normal conditions and in EMI.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 2.5-3.5 kg. EMI was produced by ligation of the anterior descending branch of the left coronary artery [10]. Macromolecular complexes of ARSases were isolated by gel-chromatography from a ribosome-free extract of rabbit liver on Sepharose 6B, as described in [1]. Activity of the ARSases was determined from the initial velocity of the *t*RNA aminoacylation reaction in the presence of saturating substrate concentrations. The composition of the reaction mixture was described in [5]. A cell-free protein-synthesizing system from rabbit liver, in a volume of 0.2 ml, contained 50 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 80 mM dithiothreitol, 0.5 mM ATP, 0.2 mM GTP, 10 mM creatine phosphate, 1 μg creatine phosphokinase, 200 μg polysomes, 10 μg *t*RNA, 60 μg protein of complexes of ARSases, 0.05 mM each of nonradioactive amino acids (excluding valine), and 0.05 mM ^{14}C-valine. The reaction mixture was incubated for 10 min at 37°C. The reaction was stopped by addition of 0.5 ml of 0.1 M KOH, and after incubation for 20 min at 37°C (to cause hydrolysis of aminoacyl-*t*RNA), 0.5 ml of 10% TCA was added to the samples. The residues were transferred to "Synpor" No. 3 nitrocellulose filters (Czechoslovakia) and washed with 5% TCA. Radioactivity was determined in toluene scintillator on a "Delta 300" counter (The Netherlands). The velocity of protein synthesis was estimated from incorporation of ^{14}C-valine into the TCA-insoluble product.

EXPERIMENTAL RESULTS

The results of the study of the composition of macromolecular complexes of ARSases of different molecular weight show that both under normal conditions and 12 h after induction of EMI they contained arginyl-, glutamyl-, isoleucyl-, leucyl-, lysyl-, and valyl-*t*RNA-synthetases (Table 1). The enzymes studied, moreover, were present in the larger amount in the complex

Department of Biological and Bioorganic Chemistry, Kaunas Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 563-565, June, 1990. Original article submitted June 27, 1989.

 0007-4888/90/0006-0750$12.50

TABLE 1. Aminoacyl-*t*RNA-Synthetase Activity in Composition of Macromolecular Complexes from Rabbit Liver under Normal Conditions (control) and 12 h after Induction of EMI ($M \pm m$, $n = 6$, $p < 0.05$)

Amino-acid specificity of ARSase	Activity, pmoles aminoacyl-tRNA/mg protein/min			
	ARSase complex, 1820 kD		ARSase complex, 840 kD	
	control	EMI	control	EMI
Arginine	793,1±27,4	956,9±22,0	93,6±7,0	252,7±16,6
Glutamic acid	388,0±40,4	593,2±19,5	89,4±4,0	181,4±18,2
Isoleucine	823,8±17,6	920,1±27,2	175,0±7,2	374,5±22,5
Leucine	1038,6±11,0	1233,8±60,5	286,5±23,2	533,0±67,7
Lysine	823,8±67,3	1105,8±51,4	93,2±15,2	295,6±54,2
Valine	434,2±17,5	318,0±5,7	108,0±20,5	280,7±23,4

TABLE 2. Incorporation of ^{14}C-Valine into TCA-Insoluble Translation Product of Endogenous *m*RNA of Cell-Free Protein-Synthesizing Systems of Rabbit Liver (in pmoles/mg ribosomal RNA, $M \pm m$, n = 14-16)

Type of protein-synthesizing system	ARSase complex, 1820 kD		ARSase complex, 840 kD	
	control	EMI	control	EMI
Homologous	4,1±0,4	4,1±0,6*	3,2±0,3	4,9±0,6
Heterologous	4,2±0,5	3,8±0,5*	3,2±0,4	4,6±0,4

Legend. Asterisk indicates that changes are not statistically significant.

with the higher molecular weight (1820 kD). The smaller complex (840 kD) is evidently a dissociation product of the heavier complex. An increase in aminoacyl-*t*RNA-synthetase activities was observed 12 h after induction of EMI in the 1820 kD complex, and a sharper increase in the 840 kD complex (Table 1). It can be tentatively suggested that one cause of this phenomenon is partial redistribution of the ARSases from the heavy into the lighter complex. This redistribution was most characteristic of valyl-*t*RNA-synthetase, activity of which was reduced in the heavy complex. Incidentally, according to data in the literature, this enzyme is least firmly associated with other ARSases in the macromolecular complex [3]. However, an alternative explanation of this phenomenon cannot be ruled out, namely the presence of factors regulating activity of the ARSases in the 840 kD complex, for example, factors such as inorganic pyrophosphatase, increased activity of which was established previously by the writers in myocardial ischemia [2].

The results suggested that changes observed in ARSase activity in the composition of the macromolecular complexes have a definite effect on the velocity of protein synthesis. This parameter was therefore studied in the next experiments in a cell-free protein-synthesizing system from rabbit liver under normal conditions and 12 h after induction of EMI. The results (Table 2) are evidence that normally the velocity of protein synthesis is higher if the heavier ARSase complex (1820 kD) is present in the system. In the case of EMI, the velocity of protein synthesis is virtually unchanged in the presence of this complex, but if the lighter ARSase complex (840 kD) is present in the system, this parameter is considerably increased.

To study the causes of this phenomenon we used heterologous cell-free protein-synthesizing systems, i.e., to a system from the liver of control animals we added one of the components isolated from the liver of the animals 12 h after induction of EMI. The results showed that polysomes and *t*RNA from the liver of the control and experimental rabbits virtually identical activity toward the velocity of protein synthesis (Table 2). The addition of ARSase complexes (840 kD) isolated from the liver of rabbits 12 h after induction of EMI to a system from the liver of the control animals, however, led to an increase in the velocity of protein synthesis.

The results are thus definite evidence of the limiting role of the macromolecular complexes of ARSases in protein biosynthesis. They also suggest that one of the mechanisms of regulation of protein biosynthesis is connected with a change in association of the ARSases into macromolecular complexes.

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