BIOPHYSICS AND BIOCHEMISTRY

Effect of Coenzyme Q10 on Proteomic Profile of Rat Plasma under Conditions of Metabolic Stress

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Proteomic profile of rat plasma was evaluated under conditions of metabolic stress (5-day starvation) and administration of coenzyme Q10 in doses of 10 and 100 mg/kg. This antioxidant and geroprotector plays a key role in cell metabolism. The expression of some low-molecular-weight proteins in response to treatment with coenzyme Q10 was observed.

Key Words: *coenzyme Q10; proteomic profile; stress*

Coenzyme Q_{10} (CoQ10) is present in all animal and human tissues. The amount of CoQ10 is maximum in body tissues with the highest energy consumption or metabolic activity, *e.g.* in the heart, kidneys, liver, and muscles [6]. The biological effects of CoQ10 are associated with its ability to undergo the reversible redox transitions. CoQ10 plays a role in the transfer of electrons and hydrogen in the respiratory chain and has a stimulatory effect on oxidative phosphorylation in the cell. CoQ10 is present in membranes of ATP-synthesizing cell structures [2,4,9]. CoQ10 deficiency is accompanied by autosomal recessive mutations, oxidative stress, and carcinogenesis. Various alimentary diseases (diabetes and cardiovascular disorders) are associated with reduced CoQ10 content [7]. Published data show that dietary consumption of CoQ10 and other antioxidants produces a geroprotective effect [3,8].

Previous studies showed that long-term dietary consumption of CoQ10 has a modulatory effect on the protein spectrum of rat blood plasma [5].

Here we studied the effect of CoQ10 on specific minor proteomic characteristics of blood plasma from rats during metabolic stress induced by starvation and subsequent complete restitution.

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MATERIALS AND METHODS

Experiments were performed on 10-week-old Wistar rats. Complete starvation (5 days, water *ad libitum*) and subsequent nutritional restitution (5 days) served as the model of metabolic stress. The animals were randomized into 5 groups of 7 specimens each. Group 1 animals (control) fed a standard diet. The rats of groups 2-5 were subjected to starvation. During the 5-day starvation period, group 4 and 5 animals orally received CoQ10 in daily doses of 10 and 100 mg/kg. The rats of groups 1, 2, 4, and 5 were guillotined after 5 days. Group 3 animals (group of recovery) received a standard diet for the following 5 days.

Before two-dimensional electrophoresis, the plasma was purified from major proteins using ProteoMiner™ microgranules (Bio-Rad). The purified samples were dissolved in a buffer containing 8 M urea, 2 M thiourea, 5% ampholines (pH 3-10), 80 mM dithiothreitol, and 16.7% solution consisting of 30% CHAPS and 10% NP40. The samples were centrifuged at 15,000*g* for 15 min. Protein concentration was measured by the

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Bradford method. Isoelectrofocusing was performed in 18-cm glass tubes with 4% PAAG (8 M urea, 4% acrylamide/methylenebisacrylamide, 2% ampholines pH 3-10, 2% ampholines pH 3-5, 3% ampholines pH 5-7, 6% solution of 30% CHAPS+10% NP40, 0.15% TEMED, and 0.03% ammonium persulfate) using a PROTEAN IEF Cell system (Bio-Rad); 100 μg total protein was layered on each tube and isoelectrofocusing was conducted under the following conditions: 100-200-300-400-500-600 V, 45 min; 700 V, 11 h; and 900 V, 2 h. After isoelectrofocusing, the tubes were placed in an equilibration buffer of 6 M urea, 20% glycerol, 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 20 mM dithiothreitol, and trace amounts of bromophenol blue. The second electrophoresis was performed in 12% PAAG in Tris-glycine buffer with cooling under the following conditions: 20 min at 20 mA per glass and than 40 mA per glass until the end of electrophoresis. The gels were stained with silver and analyzed using PDQest 8.0 software (Bio-Rad).

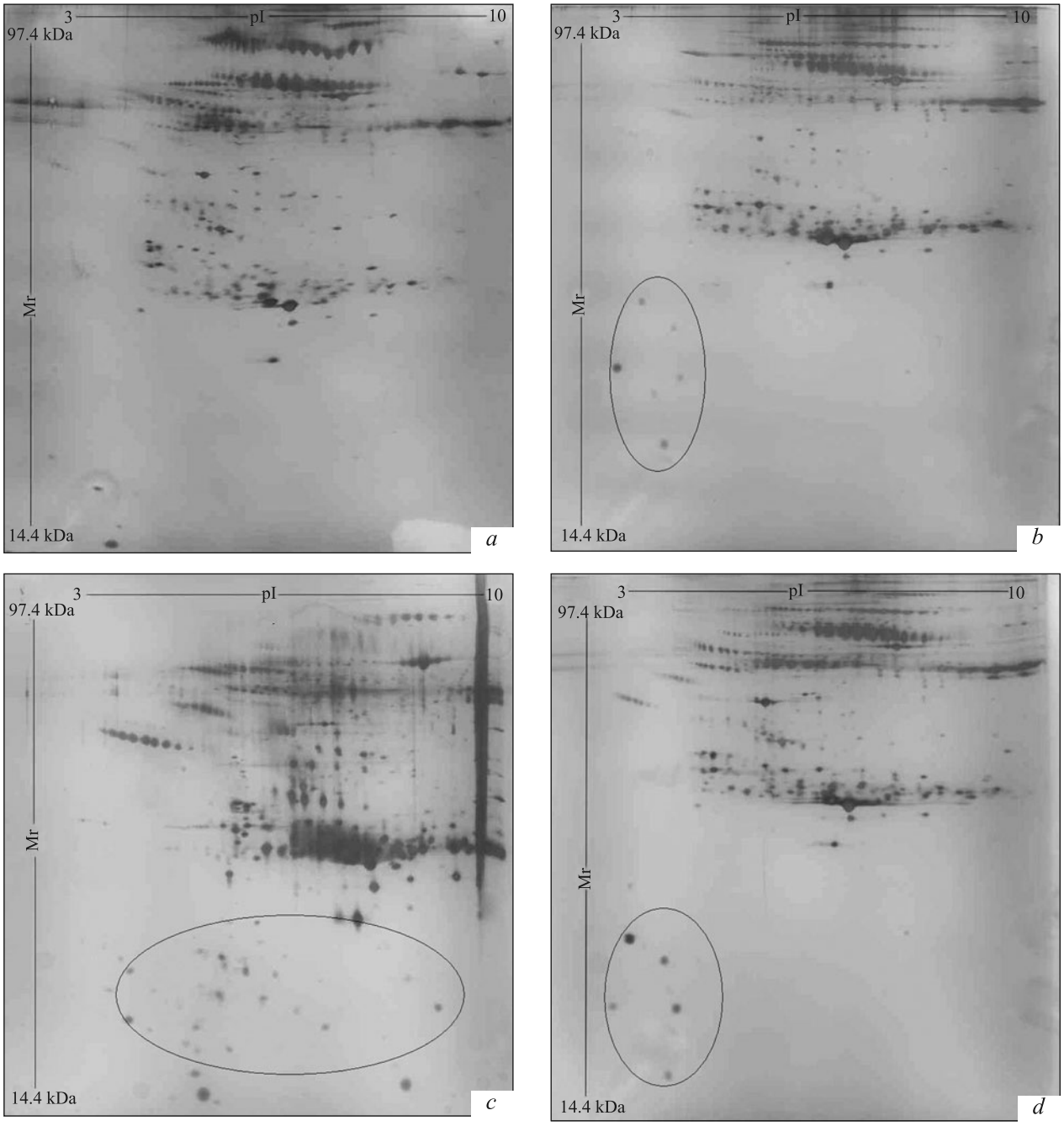

Fig. 1. Two-dimensional electrophoresis of rat plasma proteins during metabolic stress. Control group (*a*); starvation group (*b*); starvation group and 100 mg/kg CoQ10 (*c*); and recovery group (*d*). Encircled are regions of protein spots that were not found in the control group.

TABLE 1. CoQ10 Content in Rat Heart during Starvation (*M*±*m*)

Parameter	Group 1 (control)	Group 2 (5-day starvation)	Group 3 (5 day starvation and 5-day restitution)	Group 4 (5 day starvation and 10 mg/kg CoQ10)	Group 5 (5 day starvation and 100 mg/kg CoQ10)
Body weight, g before the study	154±6	152±8	157±10	153±7	155±9
after the study	215±24	148±21	224±20	160±14	170±19
Weight of the heart, g	0.82±0.11	0.64±0.06*	0.80±0,09	0.68±0.03*	0.67±0.07
Q10 in the heart, μg/mg tissue	4.30±1.16	4.24±1.65	4.48±1.54	4.85±1.75	6.95±1.95

Note. *$p<0.05$ compared to the control.

The heart tissue was homogenized in a Kinematica AG homogenizer at 1200 rpm for 90 sec. The neutral buffer consisted of 0.154 M KCl and 0.05 M Tris-HCl (pH 7.4). The homogenate was centrifuged at 5000*g* for 15 min. Butylhydroxytoluene (antioxidant, 20 mg) and isopropyl alcohol (800 μl) were added to the supernatant (200 μl). The mixture was centrifuged at 16,000*g* for 3 min. Chromatographic assay of CoQ10 in the myocardial homogenate was performed using an Agilent 1100 HPLC system and spectrometric detector. Separation was conducted in a Luna C18 analytical column (150×4.6; Phenomenex) under isocratic elution conditions (isopropyl alcohol and acetonitrile 3:2 mixture as mobile phase, 1 ml/min flow rate). Detection was performed at 275 nm.

The significance of differences was evaluated by Student's test. The differences were significant at $p<0.05$.

RESULTS

CoQ10 in a dose of 100 mg/kg contributed to the maintenance of body weight under conditions of starvation (as compared to the starvation group; Table 1). The content of CoQ10 in the myocardium was shown to increase only in rats of this group. Heart weight decreased significantly in animals of the starvation group and starvation+CoQ10 group (10 mg/kg; as compared to the control group and restitution group). A dose-dependent effect of CoQ10 was not observed at the proteomic level.

Comparison of two-dimensional protein electrophoretograms revealed differences in protein expression between the control and treated rats. The average number of protein spots in electrophoretograms (pH 3-10) was 300±28 (Fig. 1). Eleven protein spots revealed in the starvation groupwere absent in the control group (Fig. 1, *a*); 9 of these spots were focused in pl range of 5.5-6.5. The most pronounced differences were found between the control group and dietary CoQ10 group. Comparative study showed that CoQ10 consumption increased the expression of low-molecular-weight proteins (pl range 4.0-5.0). These proteins were not identified in animals of other groups. No significant differences were found between the animals receiving CoQ10 in doses of 10 and 100 mg/kg. Figure 1 shows a typical electrophoretogram. Ten protein spots (pl 4.5-6.0) were characteristic only of the restitution group (Fig. 1, *d*). A strong correlation was found between the proteomic spectra of this group and starvation group. These data indicate that metabolic stress is accompanied by the development of severe disturbances. Comparison of electrophoretograms shows that metabolic stress modulates the expression of some proteins with a molecular weight of 40-50 kDa and mean pl values of 4.5-6.5 (Fig. 1, *c*). Administration of CoQ10 over a 5-day starvation period is followed by the expression of some proteins that characterize the antioxidant function of the body. Our results are consistent with published data that CoQ10 has an adaptive effect during metabolic stress. CoQ10 reduces the percentage of apoptosis and increases the expression of Hsp25, Hsp70, and Hsp90 in hepatocytes [1].

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