Peculiarities of Rat Serum Proteome Profile in Metabolic Stress

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We identified individual proteins characterizing starvation-induced metabolic stress. Sustained changes at the level of proteome caused by metabolic stress were demonstrated. Intensified synthesis of S6 kinase ribosomal protein, immunoglobulin lambda light chains, and inter-alpha-trypsin inhibitor was observed in the experimental groups.

Key Words: *proteomics; metabolic stress; mass-spectrometry*

Proteomics implies inventory of all proteins encoded in the genome and their interactions in the organism [3]. Proteomic studies are aimed at both identification of proteins in the test organism and their functional changes. In contrast to the genome, the proteome is not constant, but is affected by external and internal factors; therefore, analysis of the proteome changes allows evaluation of these influences [1].

We previously demonstrated the effect of metabolic stress caused by starvation on the expression of some low-molecular-weight proteins in rat serum [6].

The aim of the present study was identification of proteins characterizing changes in the organism caused by acute hunger and proteins characterizing recovery after metabolic stress.

MATERIALS AND METHODS

The experiments were carried out on 10-week-old male Wistar rats. Complete starvation for 5 days (water *ad libitum*) with feeding restitution for 5 days was used as the model of metabolic stress. The animals were randomly divided into groups (n=5). Controls received complete vivarium ration, the groups of starvation and recovery were deprived of food. After 5 days, animals of the control and starvation groups were decapitated using a guillotine and the recovery group received vivarium ration for 5 days.

The proteins were separated by two-dimensional electrophoresis followed by staining with silver nitrate as described previously [6]. Trypsin hydrolysis of proteins was performed as follows: gel fragments were stained in ferrocyanide–silver thiosulfate (50 mM each) for 1-2 min, washed with deionized water (3×15 min), and incubated with 200 μl 0.1 M NH_4HCO_3 in 50% acetonitrile for 15 min at 50°C. After that, bicarbonate solution was removed and 100 μl acetonitrile was added. In 15 min, acetonitrile was removed and 3 μl modified trypsin solution in 0.05 M NH_4HCO_3 (final concentration 15 μg/ml, Promega) was added to the gel. Hydrolysis was carried out for 18 h at 37°C, then 5 μl 0.7% trifluoroacetic acid (TFA) was added and the solution was incubated for 1 h. The final solution was used for MALDI-mass spectra recording.

To this end, 0.5 μl sample and 0.5 μl 2,5-dihydroxybenzoic acid (Bruker, 20 mg/ml in 50% acetonitrile in water with 0.4% TFA) were mixed on the surface of the spectrophotometric target and dried on air.

Mass-spectra were recorded on a BRUKER Ultraflex II time-of-flight MALDI-mass spectrometer equipped with UV laser (Nd) in a reflector regimen in positive ion mode. The accuracy was not below 70 m.z.

The proteins were identified using Mascot software (www.matrixscience.com). The search in NCBI.nr

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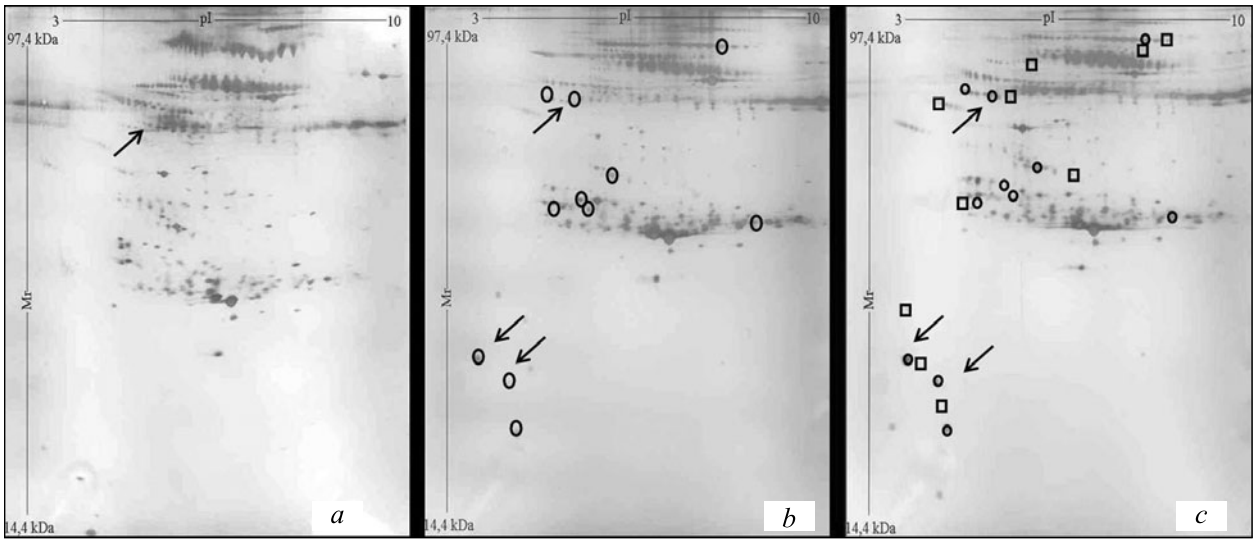

Fig. 1. Electrophoregrams of serum from rats of the control (*a*), starvation (*b*), and recovery groups (*c*). Protein spots absent in the control group (circles), characteristic of recovery group only (squares), and proteins identified by mass-spectrometry (arrows).

database was performed among proteins of all organisms considering the accuracy of peptide mass measuring not below 70 m.z. and taking into account possible oxidation of methionines and modification of cysteines with acrylamide.

Mass-spectrometry studies were performed at Human Proteome Collaboration Center, V. N. Orekhovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences.

RESULTS

Comparison of two-dimensional protein electrophoresis patterns using PDQest 8.0 software (Bio-Rad) revealed certain differences in the expression of serum proteins in rats of the control and experimental groups. The electrophoregrams (pH 3-10) usually had 300±28 protein spots (Fig. 1). Comparative study of electrophoregrams from experimental animals revealed 11 protein spots that were absent in the control group, 9 of then were located in pI range 5.5-6.5. In turn, 10 protein spots located at pI 4.5-6.0 were detected exclusively in the recovery group, the proteomic spectrum of this group maximally correlated with the proteomic spectrum of the starvation group. Mass-spectrometry showed 4 proteins, of which three proteins were absent in the control group and one was characteristic of the recovery group (Table 1).

TABLE 1. Characteristics of Identified Proteins

Protein	Incidence (number of samples)			Characteristic	Function	Published data
	control	starvation	recovery			
Ribosomal protein S6 kinase alpha-3	0	5	5	Absent in the control	Cell determination, protein synthesis, glucose homeostasis	[9]
Immunoglobulin lambda light chain	0	5	4	Absent in the control	Structural component of immunoglobulins	
Inter alpha-trypsin inhibitor, heavy chain 4, isoform CRA_b	0	5	5	Absent in the control	Plasmin inhibitor, oncomarker	[7]
Preproapo-AI	5	3	0	Absent in the recovery group	Protein translocation	[8]

Analysis of these findings indicates sustained changes at the proteome level caused by metabolic stress. In the experimental groups, enhanced synthesis of three proteins (S6 kinase ribosomal protein, immunoglobulin lambda light chains, and inter-alpha-trypsin inhibitor) was observed; moderate and low expression of preproapolipoprotein A-I was detected in the groups of starvation and recovery, respectively.

Marked expression of Hsp25, Hsp70, and Hsp90 was previously reported during starving and especially during feeding resumption; these shifts were accompanied by inhibition of apoptosis in hepatocytes [2,5]. Taking into account the results obtained in the present study we can hypothesize that the system of cell determination of protein synthesis and phenomenological peptidome pool of some functional proteins participate in adaptation to metabolic stress [4].

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