Study of Normal Human Serum Proteomic Profile under Conditions of Hyperbaric Oxygen-Nitrogen-Argon Exposure

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 1, pp. 42-44, January, 2010
Original article submitted July 2, 2009

The dynamics of changes in serum proteome was studied under conditions of experimental 9-day seclusion in a pressure chamber at 5 m H_2O pressure with modified gaseous environment. The proteomic profile for the molecular weight range of 1000-17,000 Da changed by 75%. Increased content of acute phase proteins (complement components, inter-α-trypsin inhibitor, and high-molecular-weight kininogen) was observed on day 1 of the experiment before the exposure. On day 9 of exposure, the peaks of AII, CI, and CII apolipoproteins decreased and angiotensin II peak increased.

Key Words: *hyperbaric exposure; proteomic profiling; blood serum*

The uniqueness of the proteome is determined at the genetic level, but is affected by environmental factors. Presumably, the low-molecular part of the proteomic profile (<20,000 Da) is most liable to changes in response to modulation of physiological status of the organism.

Study of plasticity of the proteomic profile under conditions of high pressure and modified gaseous mixture is expected to detect specific features of adaptation rearrangements and their impact for the health status and possibility of maintaining homeostasis under unusual conditions.

We evaluated changes in serum proteome under conditions of long-term exposure to oxygen-nitrogen-argon medium at high pressure.

MATERIALS AND METHODS

The study was carried out on blood sera from 4 volunteers aged 26-48 years admitted to participation in the experiment by expert medical committee. Preliminary procedures and methods were discussed by Committee for Biomedical Ethics of Institute of Biomedical Problems. The volunteers gave written consent to the participation in the study. During 9 days the volunteers were exposed in a deep water diving complex GVK 250 (Institute of Biomedical Problems) at a pressure of 5 m H_2O. During the first 6 days, the environment was normoxic (13.53% oxygen, 58.21% argon, 28.26% nitrogen), after which it was replaced with hypoxic mixture (9.66% oxygen, 58.21% argon, 32.13% nitrogen) for 3 days. The 9-day exposure was followed by decompression and release. Blood samples were collected 2 days before the experiment, 3 h before "diving" on day 1 of the experiment, on days 6 and 9 of exposure, and on the next day after the end of exposure. After 10 months, blood specimens were collected from the same volunteers according to the same cyclogram in order to evaluate natural variability of the proteomic profile not associated with the exposure (control).

Blood samples were left at ambient temperature for 30 min for clot formation, centrifuged at 4500*g* for 15 min without cooling, and serum aliquots were then frozen at -80°C. The proteins from serum samples were purified and concentrated using MB-WCX kits of magnetic particles (Bruker Daltonics). All the steps (pipetting of the solutions, separation of magnetic par-

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0007-4888/10/1491-0037

ticles, and application to the MALDI target) were carried out in an automated mode using ClinProtRobot 1.3 software (Bruker Daltonics). Highly purified solvents (Merck) were used. Proteomic profiles in a molecular weight range from 1000 to 17,000 Da were obtained in an automated mode using AutoXecute editor on an Autoflex III TOF mass spectrometer (Bruker Daltonics) working in a positive linear mode. Each mass spectrum was analyzed using FlexAnalysis 3.0 and ClinProTools 2.1 software (Bruker Daltonics). Quality control of all spectra was carried out the methods of multidimensional statistics (cluster analysis, Statistics 6.0) with exclusion of "overshoot" data (spectra statistically differing from the others) from the total sum of values. Further analysis was carried out using nonparametric Friedman's test (for comparison of three and more related groups).

RESULTS

A total of 149 peaks were obtained after serum treatment with MB WCX magnetic particles. Analysis by Friedman's method showed differences by 112 peaks in all volunteers under experimental conditions, while the area of only 24 peaks varied in the control. Comparison of hyperbaric exposure and control showed different amplitude of changes in the peaks. For example, on day 1 of the experiment before "diving", the area of the peak with the mass/charge (m/z) ratio of 1944 Da 5-fold surpassed the basal value, while in the control changes in the intensity of this parameter were less pronounced (Figs. 1, 2). This peak was previously identified as the fragment of high-molecular-weight kininogen [10]. The peaks areas corresponding to fragments of C3 (m/z=1348.12, 1449.06, 1562.05, and 1864.79 Da) [6,10], C3f (m/z=1777.77 and 2021.05 Da) [6], and inter-α-trypsin inhibitor (m/z=3157.14 and 4280.11 Da) also increased [5]. All these proteins are acute phase proteins characterized by different physiological properties. These proteins are characterized by high opsonizing, antiproteolytic, and bacteriostatic activities and capacity to bind free radicals and stimulate blood clotting [1]. Increased intensities of peaks corresponding to these proteins are presumably a natural reaction aimed at mobilization of the defense mechanisms and increase in the adaptation potential associated with "prestart stress" before the exposure. On day 9 of the experiment, their levels dropped below the basal levels. By the last day of the experiment, the peak of angiotensin II increased, while the peaks corresponding to apolipoproteins CI (m/z=6432.14 and 6630.19 Da) [11], AII (m/z=8669.71 Da) [8], and CII (m/z=8914 Da) [4] decreased, which could lead to inhibition of cholesterol degradation. Our results are in line with the data of biochemical analysis of the blood [3], indicating a significant increase in blood levels of triglycerides and total cholesterol, presumably related to reversible dysfunction of the liver under hyperbaric conditions.

Due to control experiment we detected the peaks characterized by high temporal and individual variability. Their changes over the 9-day hyperbaric exposure were not caused by the exposure, but were due to normal dynamics of the proteomic profile. These were the peaks of fragments of inter-α-trypsin inhibitor (m/z=2116.09 and 2272.28 Da) [10], α2-HS-glycoprotein β-chain (m/z=2740.81 Da) [7], fibrinopeptide A (m/z=1206 Da) [10], and insulin (m/z=5633.49) [9]. Changed intensity of insulin peak can be due to natural dynamics of its concentration during food digestion. It is known that insulin release is a biphasic process. The insulin secretory response of pancreatic β-cells to glucose stimulation consists in the development of phase 1, developing directly after intravenous injection of glucose. It is followed by a reduction of insulin

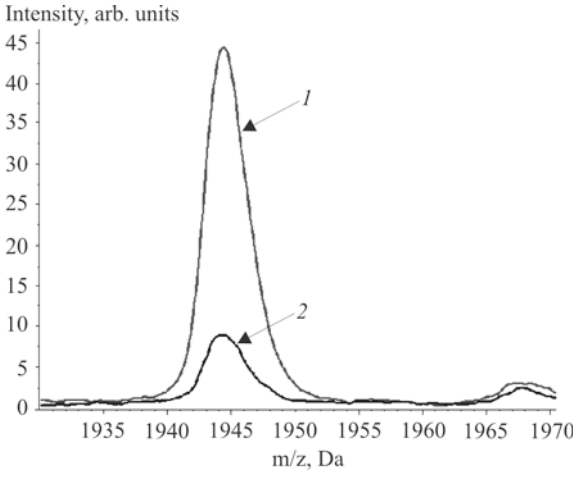

Fig. 1. Fragment of averaged spectra in experiment with 9-day hyperbaric exposure. Here and in Fig. 2: *1*) day 1 of the experiment (before "diving"); *2*) basal level (day 2).

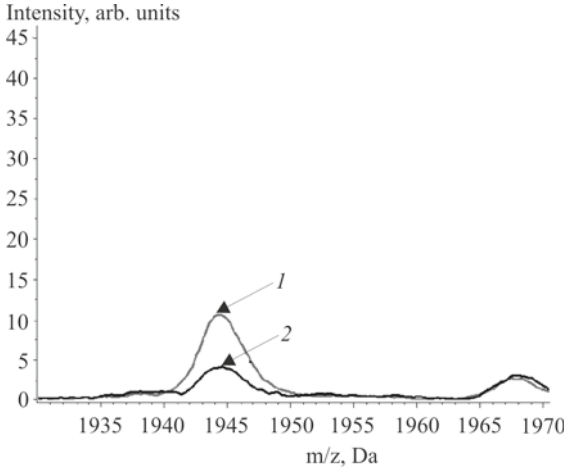

Fig. 2. Fragment of averaged spectra in the control experiment.

secretion to the lowest values and development of prolonged phase 2 of the response [2].

Hence, the proteomic profile for weights from 1000 to 17,000 Da changed by 75% over the course of 9-day seclusion in a pressure chamber. On day 1 of the experiment (before "diving"), the levels of acute phase proteins (complement components, inter-α-trypsin inhibitor, high-molecular-weight kininogen) increased, presumably because of the prestart stress before the exposure.

By day 9 of the experiment, the areas of apolipoprotein CI, AII, and CII peaks decreased and the area of angiotensin II peak increased. It is noteworthy that the detected shifts in the proteomic profile of blood serum were within the normal range, but hyperbaric exposure and modified gaseous environment formed a "violent proteome" associated with rearrangement of all body systems for adaptation to new conditions and maintenance of the homeostasis.

The study was partially supported by a grant from Leading Scientific Schools (HSh-3402.2008.4).

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