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CHARACTERISTICS OF NAD-DEPENDENT α-GLYCEROPHOSPHATE DEHYDROGENASE FROM MUSCLES OF SOME VERTEBRATES AND MAN DETERMINED BY ELECTROPHORESIS AND ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GEL

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Stable electron transport in the respiratory chain and oxidative phosphorylation in the mitochondria are maintained by preservation of the reduced state of the mitochondrial pyridine-nucleotide pool at a sufficiently high level. In muscles one of the most important systems responsible for the uninterrupted supply of hydrogen to the mitochondria [7] is the α-glycerophosphate dehydrogenase (α-GPD) system.

Two forms of α-GPD are found in mammalian cells [8]. The first is located in the cytosol and requires NAD as coenzyme (L-glycerol-3-phosphate, NAD-oxidoreductase). The mitochondrial form of the enzyme is considered [12] not to require NAD: As its coenzyme it uses flavin-adenine dinucleotide (FAD) (L-glycerol-3-phosphate acceptor-oxidoreductase). Despite many investigations devoted to the study of the molecular forms of α-GPD, the number of its isozymes and their distribution in different tissues have not yet been finally explained.

The aim of the present investigation was accordingly to study the distribution of isozymes of NAD-dependent α-GPD in the muscles of some vertebrates and man.

EXPERIMENTAL METHOD

Supramitochondrial supernatants from red (m. soleus) and white (m. quadratus lumborum) muscles of intact animals (hens, rats, rabbits) were used for analysis. Investigations of α-GPD from human muscles were carried out on autopsy material obtained from 10 persons dying from accidents at the age of between 20 and 28 years. The time between death and taking samples for analysis did not exceed 12 h. A commercial preparation of α-GPD from rabbit muscles was first dialyzed against isotonic NaCl solution for 48 h at 4°C. Mito-

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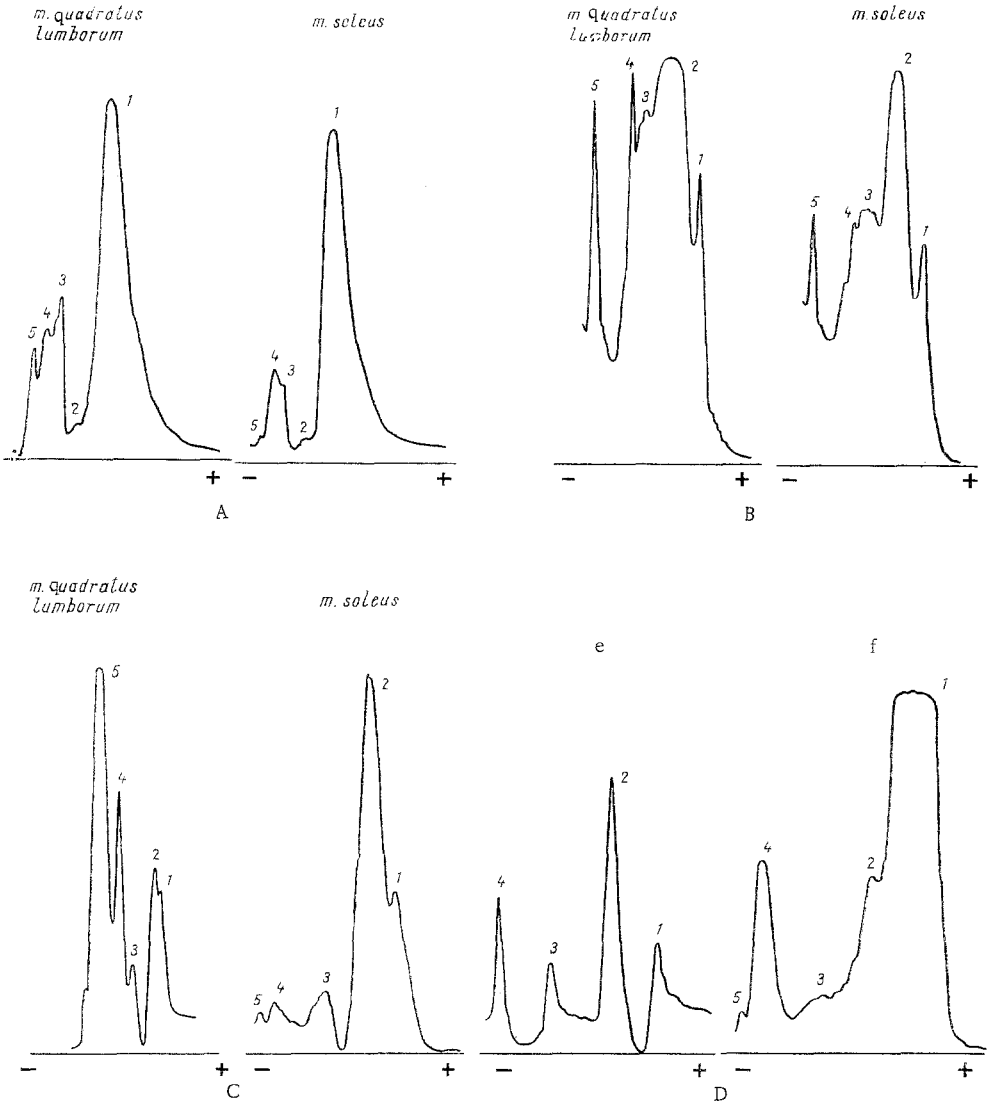

Fig. 1. Densitograms of five molecular forms of cytoplasmic α-GPD from hen (A), rat (B), and rabbit (C) muscles, and from human red muscle (m. soleus) (D, e), and crystalline α-GPD from rabbit muscle (D, f)

TABLE 1. Electrophoretic Mobility (R_f) of α-GPD Isozymes from Muscles of Some Vertebrates and Man (mean results of ten determinations)

Test subject	α-GPD isozymes, Rf				
	1	2	3	4	5
Hen	0,36	0,28	0,21	0,17	0,12
Rat	0,40	0,32	0,24	0,19	0,09
Rabbit	0,56	0,47	0,33	0,26	0,13
Man	0,55	0,41	0,24	0,12	—

chondria were isolated by differential centrifugation by the method in [11] in medium containing 0.2 mM EDTA-Na_2 and 12.5% trypsin. Isozymes of NAD-dependent α-GPD from the different sources were fractionated by electrophoresis in flat blocks of polyacrylamide gel (PAG) [13] and by isoelectric focusing with ampholines in a thin layer of 9% PAG [14] on an apparatus of our own design [5]. At the end of electrophoresis and isoelectric focusing, the localization of the α-GPD isozymes was determined by the phenazine methiosulfate–tetrazolium reaction [9]. Isoelectric profiles were obtained by removing application replicas from the gel matrix [6]. The electrophoretic and isoelectric profiles were analyzed on the ERI-65m automatic integrating densitometer.

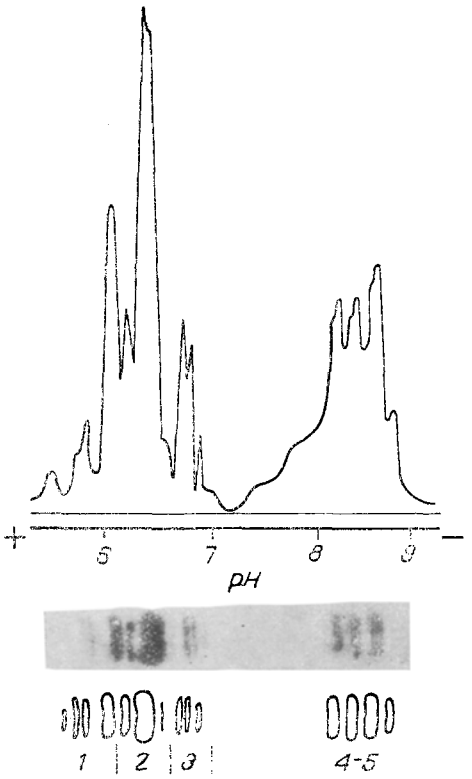

Fig. 2. Isoelectric profiles and densitograms of molecular forms of cytoplasmic α-GPD from red muscle (m. soleus) of intact rabbit. pH gradient 3.0-9.5. Duration of isoelectric focusing 140 min. Voltage 1080 V. Temperature 5°C.

Electrophoretic mobility (Rf) of the α-GPD isozymes was calculated relative to bromphenol blue, as the reference dye [3]. To rule out the possibility of nonspecific nonenzymic reactions, control development of the gels after electrophoresis and isoelectric focusing was carried out by the use of an incubation medium not containing α-glycerophosphate.

EXPERIMENTAL RESULTS

The results (Fig. 1, Table 1) show that NAD-dependent α-GPD from hen, rat, and rabbit muscles behaved on electrophoresis in PAG as five molecular forms possessing identical substrate specificity, but differing in electrophoretic mobility. Under the same conditions α-GPD from human muscles separated into four molecular forms. The results of determination of the electrophoretic mobility of the different forms of α-GPD are given in Table 1.

Analysis of the densitograms (Fig. 1) obtained under identical conditions (sensitivity of scanning 1, filter 560 nm, slit width 0.3 mm) showed conclusively that the isozyme spectrum of α-GPD in most of the tissues studied was characterized by a high content of the most mobile anodal forms (α-GPD_{1-3}), which evidently determine the total activity of the enzyme. It is generally accepted [2-4] that cytoplasmic α-GPD from mammalian muscles consists of two subunits and that during electrophoresis it separates into three active enzyme fractions. One of these fractions is more active in tissues of the brain, liver, and kidneys, another in heart and skeletal muscles. The third fraction is intermediate in activity and its content varies in different tissues. The results confirmed the existence of these three previously known molecular forms of α-GPD in the cytoplasm of vertebrate muscles. At the same time, in all the samples of muscles studied (except human muscles) the presence of two additional molecular forms of the enzyme also was discovered (Fig. 1). On the basis of their electrophoretic mobility, these forms were described as α-GPD_4 and α-GPD_5. It is interesting that the distribution of the newly discovered forms of α-GPD in red and white muscles obeys a definite rule. In red muscles (m.

soleus), differing from white (m. quadratus lumborum) both functionally and by their lower rate of glycolysis [1], their activity was considerably lower. The difference in activity of the cathodal forms of the enzyme depending on the predominant type of carbohydrate oxidation could be seen most clearly in relation to fractionation of cytoplasmic α-GPD from rabbit muscles (Fig. 1C). Changes in the isozyme spectrum of cytoplasmic α-GPD which were observed in the test samples during keeping must now be considered. For instance, on repeated electrophoresis of preparations kept for 24 h in the cold at 4°C, cathodal forms of the enzyme could be found in the form of hardly distinguishable zones of specific activity, only if the concentration of the sample under analysis was increased by 1.5-2 times. A similar picture also was observed when electrophoresis was carried out at 20°C in the absence of effective cooling of the electrode buffer solution, and consequently in the gel blocks themselves. Total inactivation of the cathodal forms of the enzyme and redistribution of activity of the anodal forms took place 48 h after the time of isolation and later during keeping. Under these circumstances activity of α-GPD_3 and α-GPD_2 was considerably reduced whereas activity of α-GPD_1 was increased. These changes evidently determine the marked differences between the isozyme spectra of native and crystalline α-GPD from rabbit muscles (Fig. 1C, D, f).

When the results of fractionation of isozymes of cytoplasmic α-GPD from red (m. soleus) muscle of rabbits by methods of electrophoresis and isoelectric focusing are compared (see Figs. 1C and 2), the existence of definite correlation between the electrophoretic mobility of individual isozymes in PAG and their distribution in the pH gradient during isoelectric focusing will be noted. Fractions of the enzymes from α-GPD_1 to α-GPD_5 occupy successive regions from the weakly acid through the neutral to the weakly alkaline region of the pH gradient. During isoelectric focusing, just as during electrophoresis, the bulk of the activity of the enzyme was accounted for by anodal forms – α-GPD_1 and α-GPD_2.

The results of this analysis of the isozyme composition of NAD-dependent α-GPD in red and white muscle of a series of vertebrates and man show that it is one of the most important enzymes regulating the utilization of key metabolites of glycolysis. Accordingly the study of the distribution of α-GPD isozymes, reflecting the velocity and direction of the α-GPD reaction, may provide an objective indicator of the changes taking place in relations between aerobic and anaerobic processes in the tissues of the body in different states.

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