The authors are grateful to M. I. Titov for providing the leu-enkephalin.

LITERATURE CITED

1. I. P. Ashmarin, Zh. Evol. Biokhim. Fiziol., 13, 570 (1977).
2. I. P. Ashmarin, in: Theoretical Bases of Pathological States [in Russian], Leningrad (1980), pp. 168-172.
3. R. I. Kruglikov, M. B. Gol'dberg, M. Ya. Maizelis, et al., Izv. Akad. Nauk SSR, Ser. Biol., No. 5, 778 (1980).
4. M. Ya. Maizelis, A. L. Zabludovskii, S. N. Shikhov, et al., Izv. Akad. Nauk SSSR, Ser. Biol. (in press).
5. C. Borghi, S. Nacosia, A. Gichetti, et al., Life Sci., 24, 65 (1979).
6. W. H. Gispen, J. van Ree, and D. de Wied, Int. Rev. Neurol., 20, 209 (1977).
7. W. Klee and M. Nirenberg, Nature, 263, 609 (1976).
8. M. Quik, L. Iversen, and S. Bloom, Biochem. Pharmacol., 27, 2209 (1978).
9. H. Rigter, Science, 200, 83 (1978).
10. K. Rozsa, L. Hirupi, and G. B. Stefano, in: Aminoergic and Peptidergic Receptors, Vol. 4 (1979), pp. 115-131.
11. D. Rudman, Endocrinology, 103, 1556 (1978).
12. G. B. Stefano and L. Hirupi, Life Sci., 25, 291 (1979).
13. F. Strand, Bioscience, 25, 568 (1975).
14. D. de Wied and W. H. Gispen, in: Peptides in Neurobiology, New York (1977), pp. 397-448.

SOME PROPERTIES OF FRUCTOSE-1,6-DIPHOSPHATE ALDOLASE FROM HUMAN MUSCLES IN ATHEROSCLEROSIS

F. N. Gil'miyarova and A. V. Babichev

UDC 612.822.1

KEY WORDS: aldolase from human muscles; isolation; properties; atherosclerosis.

The study of enzyme activity actually in human tissues in patients with atherosclerosis is very interesting. Characteristics of fructose-1,6-diphosphate aldolase (FDA), obtained in a purified form, could help to shed light on some aspects of the pathogenesis of this disease.

In the present investigation, in order to isolate and study the properties of human muscle aldolase, it was first necessary to work out a method of obtaining FDA in crystalline form, which involved the taking up of a new experimental approach based on the use of human tissue obtained at autopsy.

EXPERIMENTAL METHOD

Muscles obtained at autopsy were used to isolate FDA. The material was taken 12-18 h after death, which occurred as a result of automobile and railroad accidents. The experimental group consisted of subjects with the typical morphological features of atherosclerosis, unaccompanied by any other acute or chronic physical diseases. The control group consisted of persons with no pathological manifestations whatsoever. Using the technical approach suggested by Gulyi [1], the conditions were chosen for isolation and crystallization of FDA from human muscles. FDA was isolated in crystalline form from a common muscle extract for glyceraldehyde phosphate dehydrogenase and FDA. Maximal transfer of FDA into the residue free from ballast proteins and other enzymes took place at pH 6.0-6.25 and with a 0.55% degree of saturation with ammonium sulfate. At all stages of isolation and purification, activity of the enzyme was verified spectrophotometrically [2].

Department of Biochemistry, D. I. Ul'yanov Kuibyshev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 35-36, March, 1982. Original article submitted June 30, 1981.

0007-4888/82/9303-0267$07.50

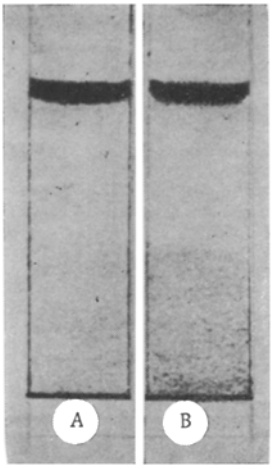

Fig. 1. Polyacrylamide gel electrophoresis (7.5% gel in Tris-glycine buffer, pH 8.6) of FDA from muscles of a normal subject (A) and patient with atherosclerosis (B).

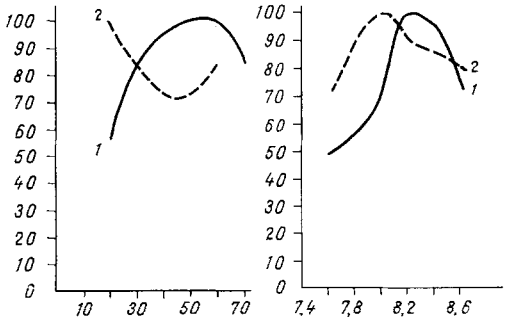

Fig. 2. Effect of temperature on activity of FDA from normal human muscles (1) and in atherosclerosis (2). Abscissa, temperature (in °C); ordinate, activity (in %).

Fig. 3. Dependence of FDA activity on pH in normal subjects (1) and patients with atherosclerosis (2). Abscissa, pH; ordinate, activity (in %).

EXPERIMENTAL RESULTS

The homogeneity of the preparations was confirmed by electrophoresis in polyacrylamide gel (Fig. 1). Differences in the properties of the enzyme in normal subjects and patients with atherosclerosis began to appear actually during the isolation process. For instance, a delay in the crystallization time of FDA was observed in the pathological material: Whereas normally it began on the 2nd-3rd day, in atherosclerosis crystals often did not begin to appear until the 10th-20th day.

Normally the crystals were bipyramidal in shape, in agreement with data in the literature [3]. In atherosclerosis they became small, although they retained their previous shape. The specific FDA activity of the muscles was high in atherosclerosis, 1.5-3 times above normal. The study of the absorption spectrum of a solution of the protein revealed differences in the pathological material within the waveband from 240 to 270 nm and 290 to 300 nm.

A study of the physicochemical properties of crystalline proteins obtained from the muscles of subjects with and without manifestations of atherosclerosis confirmed that they were

not identical, in agreement with what was observed at the crystallization stage. Determination of the temperature optimum and thermolability showed that activity of the "pathological" enzyme was reduced at temperatures of above 20°C. Under normal conditions at this temperature activity increased, to reach a maximum at 40-55°C. In atherosclerosis the enzyme was more thermolabile: Inactivation began at 60°C; in enzyme from unchanged tissue it was observed at 70°C (Fig. 2).

Investigation of dependence of FDA activity on pH showed that in atherosclerosis the pH optimum was shifted a little toward the acid side: 8.2 under normal conditions, 8.0 for the "pathological" enzyme (Fig. 3).

FDA from muscle tissue in atherosclerosis had lower substrate affinity: The value of the Michaelis constant for fructose-1-6-diphosphate increased from 3.4×10^{-6} to 4.6×10^{-6} M.

This paper thus describes the first isolation of FDA in crystalline form from the skeletal muscles of patients with atherosclerosis. The enzyme is characterized by high catalytic activity and also by changes in certain physicochemical parameters.

LITERATURE CITED

1. M. F. Gulyi, B. D. Dvornikova, A. M. Kolomiichenko, et al., in: Problems in Muscle Biochemistry [in Russian], Kiev (1954), p. 244.
2. L. T. Litvinenko, M. F. Gulyi, F. N. Gil'miyarova, et al., Ukr. Biokhim. Zh., No. 2, 214 (1972).
3. A. Dzugaj, W. Wnuk, and T. Baranowski, Acta Biochim. Pol., 21, 237 (1974).

ACTIVATION OF LIPID PEROXIDATION IN LIVER MITOCHONDRIA OF HYPERTHYROID RABBITS

A. I. Marzoev, A. V. Kozlov,
A. P. Andryushchenko, and Yu. A. Vladimirov

UDC 616.441-008.61-07:616.36-008.939.15-074

KEY WORDS: peroxidation; lipids; mitochondria; rabbit liver.

If thyroxine is added to medium containing liver mitochondria or homogenates it inhibits lipid peroxidation (LPO) in these systems [2, 5, 6, 9, 10]. Since the doses of the hormone used in these systems were close to physiological (10^{-6}-10^{-7} M) and since the antioxidant activity of the hormone was comparable with the ability of α-tocopherol to slow the rate of LPO processes [2, 10], it was suggested that thyroxine may have a specific function as a natural antioxidant [6]. It is not known, however, whether the fall in the thyroid hormone level leads to activation of LPO reactions or, conversely, to their inhibition at the expense of lipid metabolism. It has been shown that the concentration of α-tocopherol in the serum and liver rises in hyperthyroidism [12]. Meanwhile liver homogenates from hyperthyroid animals in the course of incubation accumulate products reacting with thiobarbituric acid with higher velocities than samples prepared from the liver of normal and hypothyroid animals [13]. Acceleration of LPO reactions in fragments of the sarcoplasmic reticulum in hyperthyroid rabbits also was observed by the present writers previously [3]. Direct addition of thyroxine or other derivatives of the thyroid gland, moreover, is not always accompanied by inhibition of LPO and, on the contrary, it may actually activate LPO, as has been shown in the case of isolated erythrocytes [11]. Relations between thyroid hormones and LPO are further complicated by the fact that deiodination of the hormone, considered to be essential for the realization of its biological activity, is increased when the diet is deficient in vitamin E [8], whereas elevation of the body's thyroid levels leads to an increase in the concentration of this vitamin in the serum and liver.

Biophysics Complex, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 36-38, March, 1982. Original article submitted August 7, 1981.

