ISOLATION OF CHOLESTEROL ESTERASE FROM DOG'S PANCREATIC JUICE

I. M. Karmanskii, A. L. Pichugin,
G. I. Yusupova, and Yu. A. Sysoev

UDC 577.153:612.34

KEY WORDS: cholesterol esterase; lipases; pancreatic juice.

Cholesterol esterases play an important role in the metabolism of lipoproteins [6]. Their isolation is interesting for the study of enzymic conversions of these complex proteins. However, it is difficult to obtain cholesterol esterase in the pure form, either because of the very low content of the enzyme (judging from its activity), in the aorta [3] for example, or because of the presence of large quantities of lipids in the original material which have affinity for cholesterol esterase and proteinases, in the pancreas for example. For instance, in a preparation of cholesterol esterase obtained by the writers from the pancreas by the method described in [9], both proteinases and lipases (triglyceridases) were present as impurities. The obtaining of cholesterol esterase from pancreatic juice, in which there are no substantial quantities of lipids, and in which most of the proteinases are present as zymogens, is therefore worthy of attention. Isolation of cholesterol esterase from the pancreatic juice of rats has been described [7]. However, in order to obtain sufficient amounts of juice much time and many rats are required. It was therefore decided to use dogs with a fistula of the pancreatic duct as donors of pancreatic juice in order to obtain cholesterol esterase.

EXPERIMENTAL METHOD

Pancreatic juice was obtained from a mongrel dog with a pancreatic fistula formed by Thomas' method [11]. The operation of formation of the fistula consists of securing one end of a fistula tube into the duodenum opposite the point of entry of the greater pancreatic duct. The other end of the fistula tube is exteriorized on the lateral surface of the abdominal wall and closed with a screw-fitting plastic lid. The animal after this operation loses no pancreatic juice or chyme except during the experiment and consequently remains in good condition. After the operation the dogs were kept on a balanced diet as adopted at the Institute of Nutrition, Academy of Medical Sciences of the USSR [1]. The first samples of juice were obtained one month after the operation. Samples were taken twice a week, 16-18 h after feeding. A glass catheter was introduced on these occasions through the fistula into the opening of the greater pancreatic duct. The juice thus obtained was collected in a vessel kept on ice, containing dry soy trypsin inhibitor. The juice was used at once or was kept in a frozen form at −20°C.

Biogel P-4 (50-100 mesh) from Serva, West Germany, DEAE-cellulose of the DE-32 grade, from Whatman, and HT hydroxyapatite from Bio-Rad, were used. The concentration of salts in the fractions collected on elution from the DEAE-cellulose and hydroxyapatite columns was monitored by measuring conductance on a conductometer (from Radiometer). The PHM-64 instrument (from Radiometer) was used to measure pH. The optical density of the eluates during chromatography was recorded by means of a Uvicord II continuous flow densitometer (from LKB, Sweden). The protein concentration in the eluates was determined by a modified Lowry's method, enabling solutions containing mercaptoethanol to be analyzed [2]. Peristaltic pumps from LKB and Pharmacia were used for column chromatography. Linear concentration gradients of buffer solutions were created by means of the P-3 three-channel pump from Pharmacia. Chromatographic fractions were collected by means of a Redirac collector from LKB.

Laboratory of Physical Chemistry of Biopolymers, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR. Laboratory of Physiology of Digestion, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii Meditsiny, Vol. 91, No. 3, pp. 330-332, March, 1981. Original article submitted June 24, 1980.

 0007-4888/81/9103-0344$07.50

TABLE 1. Purification of Cholesterol Esterase from Dog's Pancreatic Juice

Stage	Volume, ml	Protein, mg	Specific activity, μmoles/mg protein/h
Desalinification of juice (Biogel P-4)	13	80	0,7
Eluate with DEAE-cellulose (concentrated)	30	8,5	10
Eluate with hydroxyapatite	135	0,8	32

Cholesterol esterase activity was determined from hydrolysis of cholesteryl-1-^{14}C oleate, dispersed in glycerol with egg phosphatidylcholine and Na cholate [10]. Homogenization of the components of the substrate mixture was carried out in a glass homogenizer. The final concentration of cholesteryl oleate in the instrument was 1.38 mM. Its specific radioactivity was 2.64 Ci/mole. Lipase activity was determined by titration of free fatty acids liberated during hydrolysis of Intralipid emulsion [4]. Proteolytic activity was determined by hydrolysis of casein [8].

EXPERIMENTAL RESULTS

In the first stage of isolation of cholesterol esterase the pancreatic juice was equilibrated against 0.01 M K-phosphate buffer, pH 6.2, containing 0.1% mercaptoethanol. For this purpose, 3 ml juice was passed at the rate of 25 ml/h through a column with Biogel P-4 (1.6 × 14 cm), previously washed with this buffer solution. Protein fractions with the same conductance as the original buffer were pooled and applied at the rate of 12 ml/h to a column with DEAE-cellulose (1.6 × 40 cm), equilibrated with the same buffer solution. After application of the protein solution the column was washed with 800 ml of the original buffer, and then elution began with a linear concentration gradient of K-phosphate buffer. The total volume of eluting solution was 250 ml and the final concentration of the buffer solution was 0.3 M (this solution also contained 0.1% of mercaptoethanol). Fractions of eluate containing cholesterol esterase activity were collected, the concentration of phosphate buffer in them was increased to 0.125 M (under conductometric control 0.4 M K-phosphate buffer, pH 6.2, with 0.1% mercaptoethanol was added to them), and the resulting solution was concentrated by ultrafiltration through a PM-10 membrane. The concentrated solution (about 30 ml) was applied at the rate of 12 ml/h to a column with hydroxyapatite (1.6 × 15 cm), previously washed with 0.125 M K-phosphate buffer, pH 6.2, with 0.1% mercaptoethanol. After application the column was washed with 60 ml of the same solution, and this was followed by elution with a linear concentration gradient of K-phosphate buffer from 0.01 to 0.3 M. The total volume of eluting solution was 650 ml. Fractions containing cholesterol esterase activity were pooled and kept at 2°C.

The results of determination of cholesterol esterase activity and the protein concentration in the fractions at different stages of purification of the enzyme are given in Table 1 (the samples for determination of activity contained 1 μg protein).

The values for specific activities given in the table, it will be noted, are only approximate, for the graph of rate of hydrolysis of the substrate as a function of protein concentration in the sample was nonlinear in character. This could have been due to adsorption of the enzyme on the substrate [5].

To verify the presence of proteinases as impurities in the enzyme preparation, 5 μg of the enzyme preparation and 1% casein were incubated at 37°C for 5 h. The increase in optical density of the resulting TCA-filtrate at 280 nm was 0.02, evidence of the absence of appreciable contamination with proteinases.

Lipase impurities also were absent from the enzyme preparation. This conclusion was drawn from the fact that the volume of 0.02 M NaOH used up in titrating the lipid extract of a sample containing 10 μg of the enzyme preparation and 2% Intralipid emulsion and incubated at 37°C for 18 h did not differ from that for the control test.

On addition of desalinified pancreatic juice (50 μg protein), heated to 100°C for 3 min, to a sample containing 1 μg of the enzyme preparation and 1.38 mM cholesteryl oleate, a two-fold increase in enzyme activity was observed. This increase was not connected with the non-specific action of the protein on the enzyme–substrate system, for addition of defatted albumin from human blood serum in a quantity of 400 μg had no action on the activity of the enzyme. It can be concluded from these results that an activator of cholesterol esterase is present in the pancreatic juice.

LITERATURE CITED

1. L. S. Fomina, A. V. Popova, and Z. A. Kazakova, Fiziol. Zh. SSSR, No. 5, 588 (1967).
2. A. Bensadoun and D. Weinstein, Anal. Biochem., 70, 241 (1976).
3. P. Brecher, M. Kessler, C. Clifford, and A. V. Chobanian, Biochim. Biophys. Acta, 316, 386 (1973).
4. V. P. Dole and H. Meinertz, J. Biol. Chem., 235, 2595 (1960).
5. S. Gatt and T. Bartfai, Biochim. Biophys. Acta, 488, 13 (1977).
6. J. L. Goldstein and M. S. Brown, Annu. Rev. Biochem., 46, 897 (1977).
7. J. Hyun, H. Kothari, E. Herm, and J. Mortensen, J. Biol. Chem., 244, 1937 (1969).
8. M. Kunitz, J. Gen. Physiol., 30, 291 (1947).
9. W. E. Momsen and H. L. Brockman, Biochim. Biophys. Acta, 486, 103 (1977).
10. D. L. Severson and T. Fletcher, Atherosclerosis, 31, 21 (1978).
11. J. E. Thomas, Gastroenterology, 36, 362 (1959).

SOME CHARACTERISTICS OF SOLUBLE PROTEINS OF PACINIAN CORPUSCLES

V. L. Cherepnov and N. I. Chadaeva

UDC 612.815.1.015.348

KEY WORDS: tissue mechanoreceptors; soluble proteins; protein–carbohydrate complexes.

To study the mechanisms lying at the basis of mechanoreceptor functions information must be obtained on the biochemical processes conducted in them. Yet there is extremely little information in the literature even on the chemical composition of tissue receptors of mechanical sensation. Special attention in this direction is naturally drawn to proteins, more especially because with Pacinian corpuscles such information is limited to data on the total protein content and the content of certain amino acids. Of the proteins it is the soluble proteins that are most accessible for study. They possess the highest rate of turnover and they evidently perform chiefly metabolic functions [2].

In this connection the study of the protein composition of the medium surrounding the mechanically sensitive unmyelinated nerve ending, which has a specific ionic composition, was of definite interest and the investigation described below was aimed at its study.

EXPERIMENTAL METHOD

Pacinian corpuscles were isolated from the mesentery and pancreas of adult cats. To obtain a sufficient quantity of material the receptors were lyophilized. Water- and salt-soluble proteins were successively extracted from the homogenate with 0.9% and 10% NaCl. Gel-filtration of proteins were carried out on Sephadex G-75. The columns were calibrated with proteins of known molecular weight: trypsin, pepsin, ovalbumin, and serum albumin. The protein content in the samples was determined by Lowry's method and spectrophotometrically from absorption in the UV region. The content of nucleotides in the fractions also were

Laboratory of Cytological and Molecular Bases of Reception, Pacific Oceanological Institute, Far-Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Department of Biochemistry, N. I. Lobachevskii Gor'kii University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 3, pp. 332-334, March, 1981. Original article submitted June 5, 1980.

0007-4888/81/9103-0346$07.50