BIOCHEMISTRY AND BIOPHYSICS

CHOLESTEROL-ESTERASE OF MARROW

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Cholesterol-esterases, the enzymes which catalyze the synthesis and hydrolysis of complex cholesterol esters, are found in the liver, blood, intestinal mucosa, and in the pancreatic gland. There is no data in the literature regarding the presence of these enzymes in marrow.

In earlier work [1] it was established that the cholesterol ester content increases in the marrow of rabbits during the anemia produced by bleeding. Therefore it could be assumed that here there is some cholesterol esterase. In this work we attempted to study the cholesterol-esterase activity of marrow.

EXPERIMENTAL METHODS

Preparation of the enzyme preparation from marrow. The enzyme preparation was prepared from the red bone marrow of normal rabbits. Freshly-removed marrow from the diaphyses of the long bones of the extremities was ground with a fourfold amount of acetone. After the mixture stood 2 hours at room temperature, the acetone was drawn off and the remainder was again ground with the same quantity of a mixture of acetone and ether (1:1). After 2 hours the acetone and ether mixture was drawn off and the powder thus obtained was washed with ether. The marrow enzyme powder prepared in this way contained neither free cholesterol nor its esters. For each experiment, fresh marrow powder was always prepared.

Obtaining cholesterol esters. The esters of cholesterol – cholesterol oleate and cholesterol stearate – were obtained from cholesterol and the corresponding fatty acids by the Nedzvetsky method [2] of enzyme synthesis with pancreatic enzyme preparation. The pancreatic preparation was prepared by Vilshtetter's method. The cholesterol oleate obtained after the second crystallization from the alcohol and ether mixture had a melting point of 41° and was not precipitated by digitonin.

The cholesterol stearate which was isolated melted at a temperature of 83° and also was not precipitated by digitonin. Glycocholic acid was extracted from beef bile. In the experiment a 10% solution of sodium glycocholate was used, prepared by dissolving glycocholic acid in an equal amount of caustic soda.

Colloidal suspensions of cholesterol and its esters, prepared in the usual manner, were used as the substrates for the enzyme reaction.

In order to study the hydrolytic cholesterol-esterase activity of marrow, experiments were set up in the following way. To 2.5 ml of a colloidal suspension of cholesterol oleate (or stearate) in water, placed in a glass cylinder with a ground glass stopper, 1 ml of a 10% solution of sodium glycocholate was added. After mixing 3 minutes, 100 mg of freshly-prepared marrow powder was added, after which the sample was shaken again for 5 minutes. Then 0.5 ml of toluol was added, after shaking 10 minutes, the sample was placed in a thermostat at 37° for 72 hours. Most often two parallel samples were placed simultaneously. During the incubation, the

samples were shaken twice a day for 10 minutes. Simultaneously with the test, two control samples were set up: the first, containing a solution of cholesterol ester, sodium glycocholate, but no marrow, was set up in order to exclude the formation of free cholesterol during the incubation period from the cholesterol in the marrow powder. The pH was determined in the samples colorimetrically using a universal indicator. In all, 19 experiments were carried out – 13 with cholesterol oleate and 6 with cholesterol stearate.

After the incubation, each sample was extracted by heating it slightly with 30 ml of an alcohol-ether mixture (1 : 1). Then the cholesterol content and that of its esters was determined in an aliquot by Blur's method. The extent to which the cholesterol oleate (stearate) was hydrolyzed was measured by the increase in free cholesterol. The percentage of hydrolysis is the ratio of free cholesterol to total cholesterol.

EXPERIMENTAL RESULTS

The experiments gave only one type of results and so only some of the data are shown in Table 1. These data show that marrow has a hydrolytic cholesterol-esterase activity. In the samples with sodium glycocholate, the percentage of hydrolyzed cholesterol oleate varied between 15 and 43.9, consisting of 30.6 on the average. In the control samples, which contained cholesterol oleate, the cholesterol oleate was not split and the cholesterol ester content did not change during incubation; cholesterol and its esters were not found in the control samples, which contained marrow and oleic acid.

TABLE 1

Hydrolysis of Cholesterol Esters by Marrow. Substrate – 17 mg of Cholesterol Oleate (Sterate). Enzyme – Marrow Powder – 100 mg. Sodium Glycocholate – 100 mg. Volume of Enzyme Mixture –3.5 ml. Incubation Time – 72 Hours.

Composition of sample	Total cholesterol (in mg)	Cholesterol ester (in mg)	Free cholesterol (in mg)	Percent hydrolyzed	Total cholesterol (in mg)	Cholesterol ester (in mg)	Free cholesterol (in mg)	Percent hydrolyzed	Average percentage hydrolyzed
	Experiment No. 2				Experiment No. 5				
Cholesterol oleate + sodium glycocholate + marrow	0.82	0.58	0.24	29.2	0.60	0.37	0.23	38.3	30.6 (15.0-43.9)
Cholesterol oleate + marrow	0.85	0.60	0.25	29.4	0.56	0.36	0.20	35.7	28.3 (13.3-41.8)
Cholesterol oleate + sodium glycocholate	0.82	0.82	0	0	0.60	0.60	0	0	0
Oleic acid + sodium glycocholate + marrow	0	0	0	0	0	0	0	0	0
	Experiment No. 3				Experiment No. 4				
Cholesterol stearate + sodium glycocholate + marrow	0.61	0.40	0.21	34.4	0.64	0.50	0.14	21.9	26.2 (11.7-36.8)
Cholesterol stearate + marrow	0.65	0.44	0.21	32.3	0.60	0.45	0.15	25.0	22.6 (15.4-30.0)
Cholesterol stearate + sodium glycocholate	0.63	0.63	0	0	0.64	0.64	0	0	0
Stearic acid + sodium glycocholate + marrow	0	0	0	0	0	0	0	0	0

Similar results were obtained in experiments with the hydrolysis of cholesterol stearate. In the samples with sodium glycocholate, the percentage of hydrolyzed cholesterol stearate varied from 11.7 to 36.8, averaging 26.2.

On the basis of the results obtained, the conclusion can be drawn that cholesterol esterase exists in the marrow of rabbits, splitting cholesterol esters (cholesterol oleate and cholesterol stearate).

Cholesterol esterase, which was capable of synthesis as well as hydrolysis, was found in rat liver [3], in hog adrenal [4] and in the intestinal mucosa of rats [5].

After the presence of cholesterol esterase capable of hydrolyzing cholesterol esters was established in the marrow, the question arose whether marrow is capable of synthesizing cholesterol esters. In order to decide this problem, experiments were carried out as for the study of hydrolysis, but a colloidal suspension of cholesterol and fatty acid – oleic or stearic – in water was used as the substrate. The synthesis of cholesterol esters was measured by the amount of cholesterol esters formed. The percentage of synthesis is the ratio of cholesterol esters to total cholesterol. In all, 17 experiments were set up, 10 with oleic acid and 7 with stearic (Table 2).

TABLE 2

Synthesis of Cholesterol Esters by Marrow. Substrate – 22 mg Cholesterol, 44 mg Oleic (Stearic) Acid. Enzyme – Marrow Powder – 100 mg. Volume of Enzyme Mixture – 3.5 ml. Incubation Period – 72 hours, pH 6.0

Composition of sample	Total cholesterol	Cholesterol ester	Percentage of synthesis	Total cholesterol	Cholesterol ester	Percentage of synthesis	Average percentage of synthesis
	in mg			in mg			
	Experiment No. 4			Experiment No. 6			
Cholesterol + oleic acid + sodium glycocholate + marrow	1.36	0.46	33.8	1.00	0.13	13.0	21.03 (12.4–42.8)
Cholesterol + oleic acid + marrow	1.36	0.53	38.9	1.07	0.11	10.2	22.72 (10.7–51.4)
Cholesterol + oleic acid + sodium glycocholate (control)	1.30	0	0	1.00	0	0	
Oleic acid + sodium glycocholate + marrow (control)	0	0	0	0	0	0	
Cholesterol + oleic acid + sodium glycocholate + pancreatin	1.30	0.93	71.5	1.09	0.46	42.2	52.80 (31.7–71.5)
	Experiment No. 3			Experiment No. 2			
Cholesterol + stearic acid + sodium glycocholate + marrow	1.37	0.41	29.9	1.01	0.17	16.8	18.3 (13.8–29.9)
Cholesterol + stearic acid + marrow	1.30	0.34	26.1	1.20	0.18	15.0	18.4 (14.7–26.3)
Cholesterol + stearic acid + sodium glycocholate	1.30	0	0	1.20	0	0	
Stearic acid + sodium glycocholate + marrow	0	0	0	0	0	0	

The data presented in Table 2 indicate that cholesterol ester was found in all the samples after incubation. In the samples with sodium glycocholate, the percentage of synthesized cholesterol oleate varies between 12.4 and 42.8, averaging 21.0%.

In the control samples containing a colloidal dispersion of cholesterol and oleic acid without marrow, cholesterol esters were not found. Cholesterol esters were also not found in the samples which contained marrow and oleic acid.

Similar results were obtained during the synthesis of cholesterol stearate. The percentage of synthesized cholesterol stearate varied between 13.8 and 29.9, averaging 18.3.

It is known that the cholesterol-esterase of the adrenalin gland is distinguished by its high synthetic activity [2]. Therefore in individual experiments, the synthetic activity of the cholesterol-esterase of the marrow was compared with the activity of the adrenalin gland. Dry powdered pig adrenalin gland, prepared by Vilshtetter's method, was used as the enzyme which was added to each sample (100 mg in each). The data presented in Table 2 show that the synthetic cholesterol esterase activity of marrow is 2.5 times lower on the average than the cholesterol-esterase activity of the adrenal gland.

On the basis of the results of the work which was carried out, the conclusion can be made that cholesterol-esterase capable of synthesizing and hydrolyzing cholesterol esters is contained in the marrow of rabbits.

It is known that bile acids are necessary for the synthesis [2] and hydrolysis of cholesterol esters by the cholesterol-esterase of the adrenal gland and liver. In connection with this the question arose whether the cholesterol-esterase of marrow is activated by bile acids. In order to decide this, simultaneously with the sample containing sodium glycocholate, samples were run without it (Table 1 and 2). As is evident from the data presented, sodium glycocholate does not have an appreciable effect on the synthesis or on the hydrolysis of cholesterol esters by the cholesterol-esterase of marrow.

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