Chemical compound content and enzyme activity in supernatant and sediment of liver homogenate

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Abstract

Evaluation was performed of chemical compound contents and enzyme activities in the whole homogenate, its supernatant and sediment. Six rabbit livers were pulverized in liquid nitrogen and homogenized. After centrifugation, the contents of protein, haemoglobin, vitamin A, vitamin E, vitamin C, as well as the activities of cathepsin B, cathepsin D, superoxide dismutase, catalase, glutathione peroxidase and reductase were assessed in the whole homogenate, its supernatant and sediment. Protein, vitamin A, superoxide dismutase, catalase, cathepsin D, glutathione peroxidase and reductase reveal uniform localisation. Vitamin C and cathepsin B are localized in supernatant, whereas haemoglobin is localized mainly in sediment. Evaluation of chemical compounds and enzyme activities should be performed in the whole homogenate, supernatant and sediment to obtain a real interpretation of biochemical disturbances in the investigated material.

Key words: whole homogenate, homogenate supernatant, homogenate sediment, chemical compounds, enzymes.

Introduction

Chemical compound contents and enzyme activity are evaluated in homogenate soluble fraction [1, 2]. Its composition depends on medium type, used for homogenisation, the type of homogeniser and the method of homogenisation. Evaluation of insoluble fraction composition is omitted. However, it can also contain soluble components, which are bound in non-covalent way with cell and organelle membranes [3]. The aim of the study was to compare the contents (activities) of some components in the whole liver homogenate, as well as in its soluble (supernatant) and insoluble (sediment) fractions.

Material and methods

Six rabbit livers were used. Directly after harvesting, they were perfused with cold 0.15 mol/l NaCl until the reaction for haemoglobin with Beau reagent disappeared. The tissue samples were pulverized in liquid nitrogen in a stainless pulveriser [4]. Cold 0.15 mol/l NaCl containing 167 µmol/l BHT (1:9 w/v ratio) was added to pulverised material, which was then extracted for 30 minutes with continuous stirring at 2^0C and filtrated through gauze to remove connective tissue fibres. One part of the obtained homogenate was centrifuged (2700 x g, 30 minutes, 2^0C). The sediment was washed with cold 0.15 mol/l NaCl three times and suspended in the initial volume of this solution after the third wash.

The contents of protein [5], haemoglobin [6], vitamin A [7], vitamin E [8], vitamin C [9], as well as the activities of cathepsin B [10], cathepsin D [11], superoxide dismutase [12], catalase [13], glutathione peroxidase [14] and reductase [15] were assessed in the whole homogenate, as well as in its supernatant and sediment. The obtained results were calculated per gram of fresh tissue.

Results

The highest content of assessed compounds is present in the whole homogenate (Table 1). Protein, vitamin A and vitamin E are by half localized in supernatant and in sediment. More than

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Table 1. The content of some compounds in liver homogenate and in supernatant and sediment of liver homogenate

Compound	Homogenate	Supernatant	Sediment
Protein (mg/g of tissue)	172.8 ± 16.7	73.8 ± 6.8	93.5 ± 10.8
Haemoglobin (mg/g of tissue)	3,6 ± 0.4	0.2 ± 0.001	3.5 ± 0.1
Vitamin A (nmol/g of tissue)	56.2 ± 5.3	28.9 ± 2.3	28.8 ± 2.4
Vitamin E (nmol/g of tissue)	536.3 ± 50.4	254.6 ± 26.0	278.7 ± 29.6
Vitamin C (ng/g of tissue)	82.1 ± 3.7	80.6 ± 3.6	trace

Table 2. The activity of some enzymes in liver homogenate and in supernatant and sediment of liver homogenate

Activity	Homogenate	Supernatant	Sediment
Superoxide dismutase (IU/g of tissue)	14.8 ± 1.5	6.8 ± 0.7	6.9 ± 0.6
Catalase (IU/g of tissue)	242.6 ± 20.6	117.0 ± 16.2	115.6 ± 12.4
Cathepsin B (pNA nmol/g of tissue)	23.4 ± 1.8	21.6 ± 1.2	trace
Cathepsin D (Tyr nmol/g of tissue)	7.9 ± 0.8	3.6 ± 0.2	3.9 ± 0.2
Glutathione peroxidase (IU/g of tissue)	9.4 ± 1.1	3.1 ± 0.4	5.2 ± 0.5
Glutathione reductase (IU/g of tissue)	5.8 ± 0.5	2.2 ± 0.2	3.1 ± 0.3

70% of haemoglobin is found in sediment, whereas vitamin C is mainly present in supernatant. The highest activity of evaluated enzymes is also found in the whole homogenate (Table 2). The activities of superoxide dismutase, catalase, cathepsin D, as well as of glutathione peroxidase and reductase are localized by half in supernatant and in sediment. Cathepsin B activity is mainly present in supernatant.

Discussion

In the previously performed studies, the contents of chemical compounds and enzyme activities were assessed in the supernatants of homogenates, which were prepared in isotonic 0.25 mol/l saccharose or 0.15 mol/l NaCl solutions with or without detergent. The sage of different types of homogenisers, different homogenisation times and different centrifugation conditions lead to marked differences in the content (activity) of components in prepared supernatants. The obtained results have a limited diagnostic meaning and a limited value in the attempts to interpret biochemical disturbances.

Evaluation of the contents of chemical compounds and of enzyme activities in the whole homogenate, supernatant and sediment eliminates the above-mentioned inconveniences. Pulverisation in liquid nitrogen provides a complete disintegration of cells and cell organelles with a release of soluble components and formation of membrane structure fragments. The extract, prepared in 0.15 mol/l, is centrifuged, providing supernatant and sediment. Many cellular soluble components are bound in non-covalent way with fragments of cell membranes [3, 16]. It is then necessary to evaluate the content (activity) in the whole homogenate and in its supernatant and sediment as well. The measured content (activity) is calculated per gram of fresh or dry tissue, as well as per number of cells or mg of deoxyribonucleic acids [2, 17, 18].

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