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Dihydroceramide Desaturase Activity in Tumors

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Abstract—Dihydroceramide desaturase activity in the transplantable mouse hepatoma-22, rat hepatoma-27, M1 sarcoma, and RS1 rat cholangiocellular carcinoma has been investigated. It was found that the dihydroceramide desaturase activity in mouse hepatoma-22 is lower than that in normal mouse liver. However, the activity of this enzyme in subcutaneously and intrahepatically transplanted rat hepatoma-27 is increased compared to normal value. Dihydroceramide desaturase activity in subcutaneously and intrahepatically transplanted M1 sarcoma as well as in hepatoma-27 is dependent on the tumor microenvironment. The enzyme activity in RS1 tumor was not revealed. The data indicate that dihydroceramide desaturase activity depends on the tumor type and its microenvironment.

Key words: sphinganine, ceramide, dihydroceramide desaturase, hepatoma, tumor

Sphingolipids are involved in cell growth regulation and apoptosis. They represent one of the most diverse groups of lipid molecules in terms of the chemical structure and functional activity, which derive from a sphingoid base. The major base in native sphingolipids is sphingosine (sphingenine), an aminodiol containing 18 carbon atoms in the chain, *trans*-double bond at position 4 and having D-*erythro*-configuration. Along with sphingosine, some normal tissues also contain insignificant amounts of sphinganine (dihydrosphingosine), which lacks a double bond, being an analog of sphingosine. In comparison with homologous normal tissues, malignant tumors were found to have an increased sphinganine content in sphingolipid structure [1-6]. According to the available data, some sphinganine-containing sphingolipids, especially dihydroceramides, display a considerably lower bioregulatory effect than sphingosine-containing sphingolipids (see review [7] and the literature therein).

According to recent data on sphingolipid biosynthesis, sphinganine is a precursor of sphingosine [8]; notice that a double bond is introduced in N-acylsphinganine (dihydroceramide) by means of dihydroceramide desaturase, a recently discovered enzyme [9-13].

Since an appreciable amount of sphinganine was found in the sphingolipids from tumors, it seems therefore interesting to investigate the dihydroceramide desaturase activity in the tumors. For this purpose, dihydroceramide desaturase activity in subcutaneously transplanted mouse hepatoma-22, as well as in subcutaneously and intrahepatically transplanted hepatoma-27 and M1 sarcoma, and in RS1 rat cholangiocellular cancer, has been examined in this work.

MATERIALS AND METHODS

Synthesis of N-hexanoyl-[4,5-^{3}H]sphinganine. An amount of 2.3 mg of N-hexanoyl-sphingosine (Sigma, USA) dissolved in 0.4 ml of ethyl acetate and 6 mg of 5% $Pd/BaSO_4$ were placed in a reaction vial. The vacuum was applied until the pressure decreased to 0.1 Pa and then the vial was filled with tritium–protium mixture (1 : 49) until the pressure of 250 hPa, the reaction was performed for 135 min at room temperature under continuous stirring. After that the reaction mixture was frozen by liquid nitrogen, and the excess of tritium was removed under vacuum. The catalyst was filtered off and washed with methanol (6 × 1 ml). The filtrates

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were collected and evaporated, and the residue was dissolved in 3 ml of methanol and evaporated in order to remove the labile tritium. Washing was repeated three times. The product (activity of 7.9 mCi) was purified by HPLC (Kromasil 100 C_{18} column, 7 μm, 4.0 × 150 mm, flow rate 1 ml/min, elution by 90% methanol containing 0.05% of trifluoroacetic acid, retention time 14.52 min). The yield of labeled product was 78%; final molar radioactivity of N-hexanoyl-[4,5-^{3}H]sphinganine was 1.4 Ci/mmol. Radiochemical purity was 98%.

Preparation of tissue homogenates. Outbred rats weighing 120-150 g, which were subcutaneously and intrahepatically transplanted with hepatoma-27, M1 sarcoma and RS1 rat cholangiocellular cancer, were used for this study. Solid hepatoma-22 was transplanted subcutaneously to C3H mice weighting 18-20 g. All tumors were extracted 14-16 days after the transplantation. Liver from the healthy rats and mice was extracted the same time as tumors. Three animals were used for each experiment.

The extracted tissues were rinsed with ice-cooled 5 mM MOPS buffer, pH 7.2, containing 250 mM sucrose and 0.1% (v/v) ethanol. All further procedures were performed at 4°C.

Tissues were homogenized in a glass homogenizer using the same buffer (0.25 g tissue per ml). The protein amount in the homogenate aliquots was measured according to Lowry et al. [14].

Solubilization of the lipid substrate. An 8 mM solution of N-hexanoyl-[4,5-^{3}H]sphinganine in ethanol was diluted 10 times by dropwise addition of 9-fold excess of 5.8% BSA (Koch-Light, UK) solution in 5 mM MOPS, pH 7.2, and then sonicated for 5 min until complete dissolution of the substrate (lipid/BSA molar ratio = 1 : 1).

Determination of dihydroceramide desaturase activity. Enzyme activity was determined by a modified method [10].

An 800-μl sample of the incubation mixture containing 10 mM Tris-HCl, pH 8.5, 40 μM N-hexanoyl-[4,5-^{3}H]sphinganine (complex with BSA, 1 : 1), 50 mM sucrose, and 2 mM NADPH, was added to 200 μl of tissue homogenate.

The mixture was incubated at 37°C for 1 h. The reaction was terminated by addition of 100 μl of 8% BSA solution and 100 μl of 72% trichloroacetic acid. To remove the denatured protein, the reaction mixture was centrifuged at 6000 rpm (3600*g*) for 5 min using TsUM-1 centrifuge (Russia). The pH of the supernatant (800 μl) was adjusted to 6.0 by addition of 3.3 ml of 0.1 M Na_2HPO_4, and after that the supernatant was percolated through a Sep-Pak C_{18} cartridge in order to measure the radioactivity of tritiated water formed in the course of the reaction. Radioactivity of the eluate aliquots was determined using a TRI-CARB 2100 TR counter (Packard, USA) and a Universol Cocktail scintillation liquid (ISN Radiochemicals, Sweden).

RESULTS AND DISCUSSION

[^{14}C]Dihydroceramides [9, 11-13] or N-acyl-[4,5-^{3}H]sphinganines [10, 12, 13] are generally used substrates for the determination of dihydroceramide desaturase activity. When [^{14}C]dihydroceramides are employed as substrates, the labeled ceramides formed undergo metabolism, and the label is also included in other sphingolipids. If N-acyl-[4,5-^{3}H]sphinganines are used, all the label released after enzyme treatment is accumulated in water. For this reason N-hexanoyl-[4,5-^{3}H]sphinganine was used as a substrate in this work.

As seen in the table, the value of dihydroceramide desaturase activity in mouse hepatoma-22 is decreased compared with that of normal hepatic tissue. These data correlate with the increase of sphinganine content in sphingolipids from hepatoma-22 [3].

However, a comparison of dihydroceramide desaturase activities in normal liver and two variants of hepatoma-27 reveals the increased rates of enzyme activity in the tumor versus that of control tissue. It should be noted in this respect that dihydroceramide desaturase activity in intrahepatically transplanted hepatoma-27 exceeds that in subcutaneously transplanted. Significant differences in the enzyme activity were found in both variants of rat M1 sarcoma, but in this case the enzyme activity is higher in the subcutaneously transplanted

Dihydroceramide desaturase activity (averaged data of three replicates are given)

Tissue	Activity, pmol/min per mg protein
Mouse liver	0.47 ± 0.03
Mouse hepatoma-22	0.40 ± 0.03*
Rat liver	0.15 ± 0.01
Subcutaneously transplanted rat hepatoma-27	0.36 ± 0.02**
Intrahepatically transplanted rat hepatoma-27	0.69 ± 0.02**
Subcutaneously transplanted rat M1 sarcoma	0.48 ± 0.03
Intrahepatically transplanted rat M1 sarcoma	0.33 ± 0.02
Subcutaneously transplanted rat RS1 tumor	0.00***
Intrahepatically transplanted rat RS1 tumor	0.00***

Note: Variation in respect to control group: * $p < 0.05$; ** $p < 0.001$.
*** Activity was determined both in the presence of NADPH and NADH.

tumor. These data indicate that dihydroceramide desaturase activity is dependent on the tumor microenvironment, and increases in the case of "cognate" microenvironment. In regard to RS1 carcinoma, dihydroceramide desaturase activity is probably very insignificant, and hence could not have been determined under our experiment conditions either in the presence of NADPH nor NADH, which are the cofactors of dihydroceramide desaturase [9, 10, 12].

Appreciable variations in dihydroceramide desaturase activities in various rat tissues has been previously reported, where in some organs (heart, thymus, pancreas) it was almost negligible [13]. It is possible that the absence of enzyme activity in RS1 tumor is a result of very low activity value, if any at all, in the initial normal tissue.

Considerably higher rates of dihydroceramide desaturase activities in both variants of rat hepatoma-27 compared to that in control rat hepatic tissue (although the tumor contains increased content of sphinganine compared to control tissue) might be due to several reasons: alteration of expression, variation of desaturase complex composition, variation of preceding activities, etc. Additional investigations are needed to clarify these possibilities.

Based on the data of the present report, it is possible to conclude that the activity of dihydroceramide desaturase, an enzyme promoting the formation of the double bond at position 4 in sphinganine chain, varies in the tumors (for instance, in hepatoma) compared to homologous normal tissue (liver), and these variations are dependent on the tumor type and its microenvironment. As we found previously, the transplanted organ has an influence on tumor lipid composition [15]. The data obtained in this work demonstrate that the microenvironment also influences dihydroceramide desaturase activity.

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REFERENCES

1. Rylova, S. N., Kozlov, A. M., Kogtev, L. S., Gaenko, G. P., and Dyatlovitskaya, E. V. (1997) *Biochemistry (Moscow)*, **62**, 1053-1056.
2. Rylova, S. N., Somova, O. G., and Dyatlovitskaya, E. V. (1998) *Biochemistry (Moscow)*, **63**, 1057-1060.
3. Rylova, S. N., Somova, O. G., Zubova, E. S., Dudnik, L. B., Kogtev, L. S., Kozlov, A. M., Alessenko, A. V., and Dyatlovitskaya, E. V. (1999) *Biochemistry (Moscow)*, **64**, 437-441.
4. Kandyba, A. G., Somova, O. G., Kozlov, A. M., Zubova, E. S., Dudnik, L. B., Alessenko, A. V., Shvets, V. I., and Dyatlovitskaya, E. V. (2000) *Biochemistry (Moscow)*, **65**, 703-706.
5. Bodennec, J., Famy, C., Brichon, G., Zwingelstein, G., and Portoukalian, J. (2000) *Analyt. Biochem.*, **279**, 245-248.
6. Dyatlovitskaya, E. V., Kandyba, A. G., Kozlov, A. M., and Somova, O. G. (2001) *Biochemistry (Moscow)*, **66**, 502-504.
7. Dyatlovitskaya, E. V. (2002) *Russ. J. Bioorg. Chem.*, **28**, 3-8.
8. Huwiler, A., Kolter, T., Pfeilschifer, J., and Sandhoff, K. (2000) *Biochim. Biophys. Acta*, **1485**, 63-99.
9. Michel, C., van Echten-Deckert, G., Rother, J., Sandhoff, K., Wang, E., and Merrill, A. H., Jr. (1997) *J. Biol. Chem.*, **272**, 22432-22437.
10. Geeraert, L., Mannaerts, G. P., and van Veldhoven, P. P. (1997) *Biochem. J.*, **327**, 125-132.
11. Michel, C., and van Echten-Deckert, G. (1997) *FEBS Lett.*, **416**, 153-155.
12. Mikami, T., Kashiwagi, M., Tsuchihashi, K., Akino, T., and Gasa, S. (1998) *J. Biochem.*, **123**, 906-911.
13. Causeret, C., Geeraert, L., van der Hoeven, G., Mannaerts, G. P., and van Veldhoven, P. P. (2000) *Lipids*, **35**, 1117-1125.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
15. Kobliakov, V. A., Somova, O. G., Kondalenko, V. F., Ostashkina, N. M., Kandyba, A. G., Dubovaya, T. K., and Dyatlovitskaya, E. V. (2001) *Biochemistry (Moscow)*, **66**, 603-607.