ORIGINAL REPORT

COMPARISON OF LIPOAMIDE DEHYDROGENASE ACTIVITY IN HL-60 LEUKEMIA CELLS AND NORMAL LYMPHOCYTE

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Abstract- To determine the importance of $Q_{10}H_2$ as an antioxidant in cancer, we measured the activity of lipoamide dehydrogenase ($Q_{10}H_2$ recycling enzyme) in HL-60 and normal lymphocyte. The cultured cells of HL-60 and human normal lymphocytes were assayed in cell lysate of given number of both HL-60 and normal lymphocyte. The activity of lipoamide dehydrogenase and the protein concentration were determined by spectrometric methods. The activity of LAD was found to be 0.216µmol/min/µg protein in HL-60 cells and 0.0415µmol/min/µg protein in normal lymphocytes. Although the average protein concentration ratio in HL-60 cells to normal lymphocyte was found to be %124, the average ratio of enzyme activity in HL-60 to normal lymphocyte was %526. These results are indicative of independence of enzyme increase in HL-60 cells of increase of protein synthesis.

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Key words: Lipoamide dehydrogenase, Lymphocyte, HL-60, Leukemia

INTRODUCTION

Lipoamide dehydrogenase or LAD (dihydrolipoamide: NAD^+ Oxidoreductase, EC: 1.8.1.4, formerly EC: 1.6.4.3) is a common flavoprotein component of three multi-enzyme complex α-ketoacid dehydrogenase (namely pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and branched chain α-ketoacid dehydrogenase complexes) which is responsible for oxidative decarboxylation of α-ketoacids (1-3).

Lipoamide dehydrogenase is present in all aerobic organisms including prokaryotes, eukaryotes and Archaeobacteria. This enzyme complex is placed at the matrix surface of the inner mitochondrial membrane of all eukaryotic organisms studied to date. Moreover, there are indications that this enzyme is also present in plasma membrane.

Lipoamide dehydrogenase reduces low-molecular weight antioxidant thiols: lipoamide and lipoic acid. The latter has been proposed to have beneficial effect in treatment of diseases of the liver (3). LAD has two different subunits: ubiquinone and lipoic acid which are both important in antioxidant action (4-6). Based on previous reports, LAD is responsible for the conversion of oxidized lipoamide and ubiquinone to their reduced forms (5, 6). It is believed that this process is effective in scavenging ROS species and other radical forms that are extremely reactive to cell components. The reducing potential of reduced LAD substrates is believed to be effective in reducing the adverse growth of cancerous cells (6). While reactive oxygen species (ROS) are involved in tumor promotion, antioxidant defenses may have anti-carcinogenic effects. Activity of Glutathione peroxidase (GL-PX) and superoxide dismutase (SOD), as parts of enzymatic antioxidant systems, have been shown in several studies on leukemia and certain other cancers (5). Here, LAD was the point of focus for comparison

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between normal and Leukemic cells. Recently, a similar study has showed dramatic increase of LAD (Diaphorase) activity in rat bladder tumors (8). Nevertheless, LAD deficiencies had been recognized as the cause of several clinical manifestations, ultimately, followed by the death of the patient in early childhood (7).

MATERIALS AND METHODS

For providing the normal lymphocyte lysate, 10 ml of heparinized blood were collected from four healthy males as the control groups. The isolation of lymphocytes was performed by ficoll-paque solution by the recommended protocol of the solution. The isolated cells were removed and washed twice with phosphate buffer saline (PBS) at 2500 rpm for 15 minutes and then sonicated for 3 minutes. HL-60 cell line was provided from Iran Pasteur Institute and cultured in conventional RPMI medium (RPMI 1640 10.4g, HEPES 1.5g, $NaHCO_3$ 2g in 1000 ml deionized water, pH 7-7.2 plus 5% Fetal Calf Serum) in 37˚C with 5% CO_2. HL-60 cultured cells were then harvested, washed twice with PBS and sonicated for 3 minutes to obtain the proper lysate for the further steps.

Regarding the enzyme assay, since lipoamide and NADH are substrates of LAD the activity of lipoamide dehydrogenase was determined by measuring the oxidation of NADH in the presence of lipoamide. The activity of the enzyme present in the lysate was determined spectrophotometrically by measuring the absorbance change of the mixture (cell lysate, 0.05mM NADH, 1.5 mM EDTA, 1 mM lipoamide (Sigma), 100 mM Potassium phosphate buffer, pH 8.0) at 340 nm wavelength employing Pharmacia® UV-Visible spectrophotometer. For the absorbance coefficient of NADH (6220 $M^{-1}cm^{-1}$), we referred to re- known references of the subject. (11, 12). In order to determine the specific activity, we computed the results with the quantified total protein of the lysates. Quantification of the total protein was performed employing the method previously described by Bradford method (12).

Data were expressed as mean ± SEM analyzed by one-way ANOVA, using the SPSS software (version 11.5).

RESULTS

Lipoamide dehydrogenase specific activity was determined in lymphocyte and HL-60 cells. The mean activity of LAD in HL-60 cell line was significantly different from normal lymphocytes ($P < 0.001$). The activity of LAD was found to be 0.216 µmol/min/µg protein in HL-60 cells and 0.0415 µmol/min/µg protein in normal lymphocytes. Although the average protein concentration ratio in HL-60 cells to normal lymphocyte was found to be %124, the average ratio of enzyme activity in HL-60 to normal lymphocyte was %526.

DISCUSSION

With notion to the importance of the LAD as an antioxidant recycling enzyme, we undertook the task of studying the level of enzyme in normal lymphocytes and run a comparison. The results obtained in this study were unique in the facet that there was no similar work found in literature to date.

The enzyme assay showed a significant increase of LAD in HL-60 compared to normal lymphocytes. It is worthwhile to mention that the enzyme activities are reported per cell instead of per microgram of protein. This approach eliminated the possibility of the error that may had arose from a possible interfering effect from protein concentration that may be synthesized differently in various quantities by normal cells compared to all different kinds of cells obtained from cancer tumors.

Protein ratio in HL-60 to normal lymphocytes shows an increase of %124 while enzyme activity in similar number of cells demonstrates an increase of %526. Accordingly, it is assumed that the increase in protein synthesis does not correlate with the increase of enzyme activity in HL-60 cells. Therefore, it can be concluded that in cancerous cells, as a part of a defense mechanism the cell may act in favor of elevation of the LAD enzyme for increasing the equivalents of reductive compounds, *i.e.* reduced form of coenzyme Q_{10} as LAD substrate, of the cells to combat the oxidative stress submerging the cell toward the disease progress.

In spite of substantial increase in the rate of protein synthesis in cancerous cells, we have shown

that the increase of the lipoamide dehydrogenase activity had undergone a significant increase compared to the overall rate of protein synthesis growth in the cancerous cells. This could be indicative of that despite the important role of lipoamide dehydrogenase in cellular metabolism and growth, the increase has an exogenous cause which is the cancer condition of the cell itself. This supports the hypothesis which considers the stimulation of the expression of antioxidant recycling enzymes (*i.e.* LAD) as a defense or limiting mechanism in the cells which are in cancer state.

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Conflict of interests

The authors declare that they have no competing interests.

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