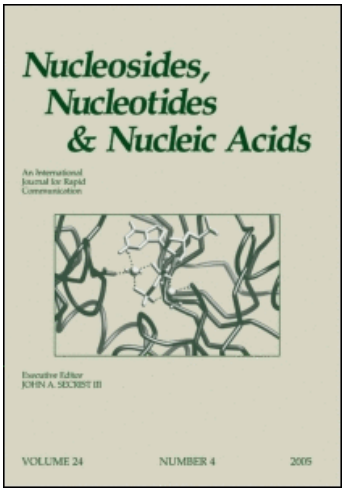

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Determination of Thymidine Phosphorylase Activity in Human Blood Cells and Fibroblasts by a Nonradiochemical Assay Using Reversed-Phase High-Performance Liquid Chromatography

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DETERMINATION OF THYMIDINE PHOSPHORYLASE ACTIVITY IN HUMAN BLOOD CELLS AND FIBROBLASTS BY A NONRADIOCHEMICAL ASSAY USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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□ *In this study, we demonstrated that the highest activity of thymidine phosphorylase (TP) was found in peripheral blood mononuclear (PBM) cells followed by that of thrombocytes and granulocytes whereas no activity of TP could be detected in erythrocytes. The activity of TP in leukocytes proved to be intermediate compared to the TP activity observed in PBM cells and granulocytes. The activity of TP also was readily detectable in human fibroblasts.*

Keywords Thymidine phosphorylase; MNGIE; Blood cells

INTRODUCTION

Thymidine phosphorylase (TP) catalyzes the first step in the degradation of the pyrimidine deoxynucleosides thymidine and deoxyuridine. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disease which is caused by a thymidine phosphorylase deficiency.[1] Clinically, MNGIE is characterised by ptosis, progressive external ophthalmoplegia, severe gastrointestinal dysmotility, cachexia, peripheral neuropathy, and skeletal myopathy.[1] In patients with MNGIE, no or a severely reduced TP activity was detected in leukocytes which was accompanied by the presence of strongly elevated levels of thymidine and deoxyuridine in plasma.[1] Recently, we developed a sensitive and fast assay of the TP activity in leucocytes based on the separation of thymine

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and thymidine using reversed-phase HPLC.[2] In this study, we investigated whether the TP activity can be detected in other blood cells and fibroblasts as well.

MATERIALS AND METHODS

Peripheral blood mononuclear (PBM) cells (lymphocytes and monocytes) were isolated from 15 ml EDTA-anticoagulated blood by centrifugation over lymphoprep and the cells from the interface were collected and treated with ice-cold NH_4Cl to lyse the contaminating erythrocytes.[3] Granulocytes were isolated from the pellet of the centrifugation step over lymphoprep by lyses of the erythrocytes with ice-cold NH_4Cl.[3] Platelets were purified from the supernatant (platelet-rich plasma) obtained after density gradient centrifugation of the blood sample. Leukocytes (lymphocytes, monocytes, and granulocytes) were isolated from 4 ml of EDTA blood, as described before.[2] Erythrocytes were isolated from 2 ml of EDTA blood by centrifugation ($250\times g$, 10 minutes) and the plasma and upper layer of the resulting cell pellet, containing contaminating leukocytes, were removed. The erythrocytes were washed 3 times with phosphate-buffered saline and collected by centrifugation ($650\times g$, 10 minutes).

The TP assay was performed in a reaction mixture containing an aliquot of cell sample, 35 mM potassium phosphate (pH 7.4), 1 mM dithiothreitol, and 2 mM thymidine in a total volume of 0.5 ml. The reaction was started by the addition of the sample. After 15, 30, 45, and 60 minutes, 100 μl was removed from the reaction mixture and the reaction catalysed by TP was terminated by the addition of 11 μl of ice-cold 8 M $HClO_4$ and kept on ice for 10 minutes. After centrifugation, the resulting supernatants were saved for analysis by reversed-phase HPLC, as described before.[2] The supernatant (75 μl) was injected into the HPLC system and separation of thymine from thymidine was performed isocratically [0.2% (w/v) acetic acid and 7% (v/v) acetonitrile] at a flow rate of 1 ml/min by HPLC on a reversed-phase column (Alltima C18 rocket, 250 × 4.6 mm, 3 μm particle size, Alltech Associates Inc., Deerfield, IL) and a guard column (Alltima C18 5 μm particle size) with online UV detection at 265 nm. The retention times of thymine and thymidine were 2.5 and 3.6 min, respectively.

RESULTS

To investigate the activity of TP in human blood cells we purified the various blood cell types with density centrifugation from four individuals. The highest specific activity of TP was observed in PBM cells (mean ± SD; 526 ± 286 nmol/mg/h) followed by that of thrombocytes (374 ± 55 nmol/mg/h) and granulocytes (270 ± 168 nmol/mg/h). The specific

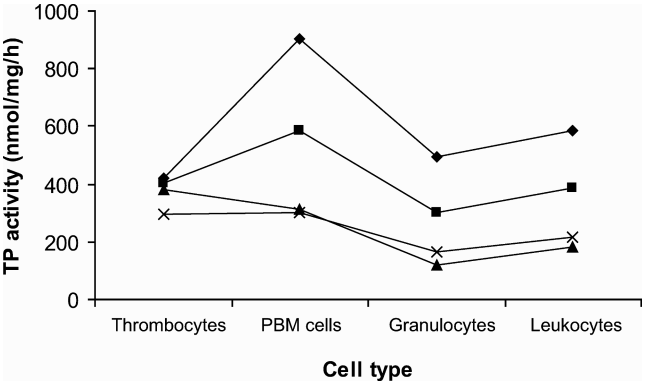

FIGURE 1 TP activity in human blood cells. The blood cells were purified from 4 individuals using density gradient centrifugation.

activity of TP in leukocytes (342 ± 185 nmol/mg/h) was in between that of PBM cells and granulocytes. In erythrocytes, no activity of TP could be detected at all. Figure 1 shows that in case a high, intermediate or low TP activity is present in PBM cells of an individual, it is paralleled by a high, intermediate, or low TP activity in other blood-cell types of the same invidual as well. The activity of TP was also readily detectable in human fibroblasts (88 ± 33 nmol/mg/h, $n = 3$).

DISCUSSION

TP or platelet-derived endothelial-cell growth factor plays an essential role in the homeostasis of thymidine and the process of angiogenesis. In this study, we demonstrated that the highest activity of TP was found in PBM cells followed by that of thrombocytes and granulocytes whereas no significant activity of TP could be detected in erythrocytes. The activity of TP in leukocytes proved to be intermediate compared to the TP activity observed in PBM cells and granulocytes. It has been shown that TP is highly expressed in human monocytes and macrophages, which is in line with the high TP activity in PBM cells.[4] In general, a large variation was observed for the TP activity in PBM cells, granulocytes, and leukocytes of different individuals. In contrast, the variation in the TP activity in thrombocytes obtained from four individuals was small. It has been shown that the mRNA and protein expression of TP is upregulated by several cytokines and interferons.[4–6] In particular, major inflammatory cytokines are associated strongly with TP expression which might be responsible for the high TP activity observed in some individuals. The fact that the TP activity was readily detectable in all human blood cells, except erythrocytes, and fibroblasts indicates that these cell types could be used for the diagnosis of patients suffering from MNGIE.

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