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β-Hexosaminidase isoenzyme profiles in serum, plasma, platelets and mononuclear, polymorphonuclear and unfractionated total leukocytes

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Abstract

Objectives: The relative proportion in percentage of the isoenzyme A of β-hexosaminidase (Hex) is the single discriminatory function most frequently used for the biochemical screening of heterozygote Tay–Sachs disease carriers. It has been suggested that the assay of the Hex isoenzymes in homogeneous cell preparations is preferable to that in mixed total leukocytes which present greater interindividual variation. The major aim of our study was the evaluation of this hypothesis.

Design and methods: Total Hex and its Hex A and Hex B isoenzymes were determined in different samples of serum and plasma (n = 81) as well as in lysates of platelets (n = 75), and mononuclear (n = 81), polymorphonuclear (n = 81) and mixed total leukocytes (n = 33).

Results: The interindividual variations found for % Hex A in the different biological samples were: plasma (CV = 23.4%), platelets (CV = 10.2%), mononuclear (CV = 5.7%), polymorphonuclear (CV = 5.3%) and total leukocytes (CV = 7.1%). Although the relative proportion of Hex A was significantly greater in polymorphonuclear than in mononuclear leukocytes ($P < 0.001$), a statistical significance was not attained for the correlation between the relative proportions of blood polymorphonuclear cells and Hex A in mixed total leukocytes (r = 0.220).

Conclusions: The use of total leukocyte lysates does not appear to introduce a significant increase for the interindividual variation of the Hex A isoenzyme relative proportion in relation to the use of homogeneous cell preparations.

Keywords: β-hexosaminidase isoenzymes; Tay–Sachs disease; Platelets; Mononuclear; Polymorphonuclear; Total leukocytes; Sandhoff's Disease; Serum; Plasma; Diabetes

Introduction

Although β-*N*-acetylhexosaminidase (Hex, EC 3.2.1.52) is the recommended trivial name, this lysosomal enzyme is still referred to as β-hexosaminidase [1]. Two main isoenzymes have been characterized: Hex A formed by α and β subunits, and Hex B, only formed by β subunits. An inability to metabolize the GM2 ganglioside, in which the Hex A isoenzyme intervenes, is the origin of a series of lysosomal diseases (GM2 gangliosidosis), which are of recessive autosomal type [1]. Mutation of the gene that encodes the α subunit leads to a deficiency of Hex A isoenzyme in Tay–Sachs disease (TSD), while the mutation of the gene that encodes the β subunit leads to a deficiency of both Hex A and Hex B isoenzymes in Sandhoff's Disease (SD).

In the laboratory, the diagnosis of homozygote patients and screening of heterozygote carriers were first carried out with enzymatic tests, and more recently were extended to incorporate DNA-based procedures for the identification of specific mutant alleles. However, the DNA-based analysis, which would have poor sensitivity in general TSD screening of the non-Jewish population [2] is currently the most useful as an adjunct to biochemical testing. Enzymatic methods, when suitably chosen, may detect nearly all of the clinically important mutations with a diminished Hex synthesis, stability or catalytic efficiency [2].

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Most laboratories use the single discriminatory function of relative Hex A proportion as a percentage in order to define a TSD heterozygote range, a normal range and an inconclusive zone between the above groups [3]. Serum Hex testing may be used for heterozygote carrier screening [2,4,5], although oral contraceptives, pregnancy and liver disease increase the proportion of serum Hex B [2,4–6], and may be important causes of inaccurate assignment. Any individual whose serum test is inconclusive requires analysis of the Hex isoenzymes in peripheral leukocyte lysates [2,3,7]. However, as the specific activity of the Hex and the relative proportion of its isoenzymes are significantly different in lymphocytes, monocytes and granulocytes, it has been suggested that the use of an homogeneous cell population may be preferable to a mixed total leukocyte preparation for identifying TSD carriers [8–10]. Consequently, the determination of Hex isoenzymes in mononuclear (MN) and polymorphonuclear (PMN) leukocyte populations [9,10] or in platelets [8,11] has been proposed for TSD screening with a lesser intra- and interindividual variation of the Hex A relative proportion.

The aim of our study was to determine Hex isoenzyme profiles in serum, plasma, as well as in lysates of platelets, MN, PMN and mixed total leukocytes from non-TSD subjects, using a thermodynamic procedure.

Materials and methods

Blood samples were collected in Vacutainer tubes (BD Vacutainer Systems, Plymouth, England) without additives for serum separation, and with EDTA-K3 to separate the plasma, leukocytes and platelets. The total leukocyte, MN and PMN populations were isolated using Polymorphoprep solution (Axis-Shield PoC As, Oslo, Norway) as previously described [9,12]. The platelets were isolated according to the procedure described by Levy-Toledano et al. [13]. The leukocyte and platelet pellets obtained were resuspended in 350 μL of 9.0 g/L NaCl solution, and the suspension was frozen at −36°C for at least 24 h; this was thawed and sonicated in a Sonopuls ultrasonic homogenizer (Bandelin Electronic, Berlin, Germany). After centrifugation for 15 min at 10,000 × *g*, the supernatants were assayed for protein concentration using the pyrogalol red-molybdate method in a Dimension RxL analyzer (Dade Behring, Mosburg, Germany), and the Hex specific enzyme activity was expressed in International Units per g protein (U/g). Hex activity in serum, plasma, and platelet and leukocyte lysates was measured with sodium-3,3′-dichlorophenylsulfonphthaleinyl-*N*-acetyl-β-D-glucosaminide as substrate, using reagents commercialized by Shionogi and Co (Osaka, Japan). With this substrate, the enzyme's apparent activation energy is directly related to the relative proportion of the Hex isoenzymes, as the Hex A has an activation energy significantly lower than that of the Hex B [14,15]. The enzyme activities were determined in duplicate at 25°C, 30°C, 35°C and 37°C in a Cobas Bio analyzer (Roche Diagnostics, Basel Switzerland), and the slopes of the Arrhenius plots and the activation energy were calculated as previously described for the determination of the Hex isoenzyme profiles in serum/plasma [14], and platelet and leukocyte lysates [9,12].

Statistical analysis of the data was carried out using the Microsoft Excel (v.5.0) package. The Kolmogorov–Smirnov test was applied to check for normality. Parametric tests (Student's *t* test and Pearson's correlation coefficient) were used when the data had Gaussian distribution; otherwise, non-parametric tests (Mann–Whitney *U* test and Spearman's correlation coefficient) were used. Lineal regression analysis was performed using the Passing–Bablock method. The results were expressed as mean ± SD (median), and statistical significance was accepted as a P value of less than 0.05.

Results

In 81 non-TSD individuals of both sexes (healthy controls, pregnant women and patients with different diseases), Hex activities were determined in samples of serum and plasma. A high correlation was found between the values ($r = 0.953$, $P < 0.001$), although the enzymatic activity in serum was significantly higher than in plasma ($P < 0.001$) as shown in Fig. 1A. The difference in serum/plasma activity had a highly significant correlation with the platelet count ($r = 0.846$, $P < 0.001$). No statistically significant difference was found between the apparent activation energies of Hex in serum and plasma, with the values having a high correlation between each other (Fig. 1B).

These results suggest that although the activities of total Hex and its isoenzymes are higher in serum than in plasma, the relative proportion of the Hex A and Hex B isoenzymes is not modified, as shown in Table 1. However, the ratio of enzymatic activities of the Hex in serum/plasma did present significant correlations with the ratio of the activation energies in serum/plasma ($r = 0.303$, $P < 0.01$), and with the ratio of relative proportions of Hex B in serum/plasma ($r = 0.257$, $P < 0.05$). This would suggest that the larger the proportional increase of enzymatic activity in serum is in relation to plasma, the relative proportion of Hex B in serum would also have to increase.

Table 2 shows the results obtained for Hex and its isoenzymes in platelets in 31 healthy control subjects, 23 pregnant women and 21 diabetic patients. Although significant differences were occasionally found for specific enzymatic activities, in the case of the relative proportion of Hex A isoenzyme no significant differences were found for the groups of pregnant women and diabetics in relation to the control group (Table 2). In the total group ($n = 75$), no significant correlation was found between the mean platelet volume and the specific activity of total Hex ($r = -0.115$),