Plasma β -N-Acetylhexosaminidase Isoenzyme Composition and Temperature Conversion Factors

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Using the chromogenic substrate 3,3'-dichlorophenylsulfonphthaleinyl-N-acetyl- β -D-glucosaminide for the activity determination of plasma β -N-acetylhexosaminidase (Hex), the temperature conversion factors (TCF) offer a highly significant positive correlation with the relative proportion of Hex B isoenzyme ($P < 0.001$). The calculation of TCF 37°/25°C allows the isoenzyme composition

of Hex to be determined quickly and cheaply. The results may be superimposed over those obtained in a previously described method based on the calculation of the enzyme's activation energy using four temperatures. However, the use of TCF 37°/30°C does not appear to comply with the required demands. J. Clin. Lab. Anal. 14:327–329, 2000. © 2000 Wiley-Liss, Inc.

Key words: plasma β -N-acetylhexosaminidase; isoenzymes; temperature conversion factors

The lysosomal β -N-acetylhexosaminidase (Hex, E.C. 3.2.1.52) is a group of glycoprotein isoenzymes that differ in their subunit composition. The isoenzymes Hex A (composed of α and β polypeptide chains) and Hex B (composed only of β chains) are the two major forms (1,2). The so-called intermediate forms are post-translational modifications. Several authors have demonstrated the interest of determining Hex activity in serum/plasma in different physiopathological situations, and this interest increases when its isoenzyme composition is studied (3–10).

We have recently developed a thermodynamic method that permits the determination of Hex isoenzymes in different biological specimens (11,12). The method is based on the fact that, toward the chromogenic substrate 3,3'-dichlorophenylsulfonphthaleinyl-N-acetyl- β -D-glucosaminide (CPR-NAG), the activation energy of the Hex B isoenzyme ($E_a \approx 75.1$ kJ/mol) is significantly higher than that of Hex A ($E_a \approx 41.8$ kJ/mol) (11,13). The analytical process, carried out in a Cobas Bio analyzer, determined the activity of Hex in duplicate at four temperatures (25°, 30°, 35°, and 37°C), thus enabling the activation energy to be calculated with suitable precision (CV < 1.4%).

With a view to a possible simplification of the technique, and its adaptation to analyzers, such as the Hitachi 717, which allow two temperatures to be selected (30° or 37°C), an evaluation was made about the determination of the isoenzyme composition of plasma Hex by calculating the temperature conversion factors (TCF) 37°/30°C and 37°/25°C.

MATERIALS AND METHODS

Blood samples were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing K₃EDTA as antico-

agulant. The determination of plasma Hex activity using CPR-NAG as substrate and the thermodynamic evaluation of its isoenzyme composition with regard to the calculation of its activation energy have been described in a previous communication (11). The commercially available reagent NAG Rate Test from Shionogi and Co. (Osaka, Japan) was used, and for the preparation of reagent solution 8.2 ml of distilled water was added to each bottle containing lyophilized substrate and buffer. The reagent was prepared and the analyzers were programmed in such a way that the manufacturer's specifications for the manual technique were maintained.

The Cobas Bio centrifugal analyzer (Roche Diagnostics, Basel, Switzerland) was programmed as follows: calculation factor, 1,029; type of analysis, 2; wavelength, 575 nm; sample volume, 10 μ l; diluent volume, 50 μ l; reagent volume, 150 μ l; incubation time, 0 sec; time of first reading, 300 sec; time interval, 30 sec; number of readings, 10; blanking mode, 1; printout mode, 2.

The Hitachi 717 analyzer (Roche Diagnostics) was programmed as follows: analysis method [Cint-A] [28] [50]; sample volume [12.5] [6]; R1 volume [250] [20] [No]; R2 volume; [0] [20] [No]; wavelength [575]; calculation factor [9,473]; Sd limit [0.1]; duplicate limit [1,000]; sensibil. limit [0]; abs. limit (In/De) [9,000] [Incremen].

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Fig. 1. Correlation and regression between the proportion as a percentage of Hex B isoenzyme and the TCF 37°/30°C (A) and TCF 37°/25°C (B) obtained in the Cobas Bio analyzer.

The calculation factors programmed in the two analyzers were established with regard to the enzyme activity at 37°C obtained for the Stand NAG Control (Shionogi and Co.) reconstituted with 1 ml of 45 g/l Ektachem bovine serum albumin solution (Johnson and Johnson Clinical Diagnostics Inc.) as described in a previous paper (11).

Statistical analysis of the data was carried out using the SPSS package. The Kolmogorov–Smirnov test was performed to check for normality. The significance of differences between the values was evaluated by the Wilcoxon sign range test for matching data. Linear regression analysis was performed using the Passing–Bablok method and Spearman's

Fig. 2. Correlation and regression between the activities at 30°C (○) and 37°C (●) obtained for plasma Hex in the Cobas Bio and Hitachi 717 analyzers (A) and between the proportion of Hex B isoenzyme and the TCF 37°/30°C obtained in the Hitachi 717 analyzer (B).

correlation coefficient was used. The clinically acceptable value for the standard error of the estimate was established with regard to the reference range for the proportion of plasma isoenzyme Hex B obtained in our laboratory (18–40%), according to previously published criteria (14).

RESULTS

Figure 1A shows the correlation and regression found between the TCF 37°/30°C of plasma Hex obtained in the Cobas Bio analyzer and the proportion as a percentage of Hex B isoenzyme. The standard error of the estimate is higher

than the clinically acceptable value ($S_{y,x} \approx 1.4\%$). Figure 1B shows the results obtained by using TCF 37°/25°C, which offer a greater correlation coefficient and less dispersion, with a standard error of the estimate around the clinically acceptable value.

The activities at 30° and 37°C obtained for the Hex in 58 plasma samples in the Cobas Bio and Hitachi 717 analyzers show a high correlation coefficient, and the difference between the means does not reach statistical significance (Fig. 2A). However, the TCF 37°/30°C calculated using the activities obtained in both analyzers shows a worse correlation coefficient ($r = 0.905$, $P < 0.001$). Figure 2B shows the results obtained by correlating the TCF 37°/30°C from the Hitachi 717 analyzer with the proportion as a percentage of isoenzyme Hex B. The standard error of the estimate is above the clinically acceptable value.

DISCUSSION

The results of the study of the enzyme heterogeneity of Hex by determining its activation energy fully agree with those obtained using thermal inactivation or electrophoretic methods or by using specific substrates for the HexA isoenzyme (11,12).

Considering the data obtained, it is possible to conclude that by calculating the TCF 37°/25°C, it is possible to determine the isoenzyme composition of plasma Hex with results that are similar to those obtained by mean the calculation of its activation energy using four temperatures. The use of TCF 37°/30°C leads to poorer results, as its correlation with the proportion as a percentage of Hex B is less, and the values are more widely dispersed (Fig. 1).

The determination in duplicate of the enzyme activities in the Hitachi 717 may reduce the dispersion obtained by correlating the TCF with the proportion of Hex B isoenzyme (Fig. 2B) and give results similar to those obtained with the Cobas Bio (Fig. 1A). In any case, the use of TCF 37°/30°C does not appear to meet the minimum requirements for determining Hex isoenzymes, as the standard errors of the estimate are much higher than the clinically acceptable value. On the contrary, the calculation of TCF 37°/25°C enables a simple and economic evaluation of the enzyme heterogeneity of plasma Hex.

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