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Clinica Chimica Acta 355 (2005) 55–60

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Thermodynamic study of β-*N*-acetylhexosaminidase enzyme heterogeneity in human seminal plasma

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Received 27 October 2004; received in revised form 8 December 2004; accepted 8 December 2004

Abstract

Background: It has been suggested that the activity of β-*N*-acetylhexosaminidase (Hex) in seminal plasma may be used as a biochemical marker of azoospermia. The purpose of our study was to evaluate this hypothesis using a thermodynamic procedure developed to determine total Hex activity and that of its isoenzymes in this biological fluid.
Methods: Using the substrate 3,3′-dichlorophenolsulphoftaleinil *N*-acetyl-β-D-glucosaminide, a highly significant difference ($p<0.001$) is found between the activation energy of Hex A (41.5 kJ/mol) and of Hex B (72.3 kJ/mol), making it possible to determine the activity of these isoenzymes from the apparent activation energy of the total Hex in seminal plasma.
Results: A significant difference between the normozoospermic and azoospermic groups was only found for Hex A isoenzyme activity ($p<0.05$), although with considerable overlapping between the values of both groups. Significant partial correlations were found for the total Hex, Hex A and Hex B activities with the immobile spermatozoa count ($p<0.01$) and for total Hex and Hex B with the dead spermatoza count ($p<0.05$). In turn, Hex A had a significant partial correlation with the live spermatozoa count ($p<0.05$); however, Hex activity in seminal plasma of acromosomal origin appears to be of little importance in quantitative terms.
Conclusions: It was not possible to confirm that total Hex activity in seminal plasma, or even of its isoenzymes Hex A and Hex B, is a suitable biochemical marker of azoospermia (efficiency≤67%). The thermodynamic procedure described may be a useful alternative for the study of the Hex enzyme heterogeneity in spermatozoa.

Keywords: Seminal plasma; β-*N*-Acetylhexosaminidase isoenzymes; Activation energy; Heat inactivation; Azoospermia; Oligozoospermia; Normozoospermia

1. Introduction

The lysosomal glycosidase β-*N*-acetylhexosaminidase (Hex, EC 3.2.1.52) has two major isoenzymes: Hex A, composed of α and β polypeptide chains, and Hex B composed only of β chains [1]. Although

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doi:10.1016/j.cccn.2004.12.015

Kapur and Gupta characterised both isoenzymes in human seminal plasma [2], later studies only focused on total enzymatic activity [3–6], suggesting its use as a possible biochemical marker for azoospermia [4].

Studies of the apparent enzyme activation energy have provided interesting data with respect to the heterogeneity of different enzyme systems [7–11]. In the case of Hex, this thermodynamic variable, using the chromogenic substrate 3,3′-diclorophenolsulphoftaleinil *N*-acetyl-β-D-glucosaminide, is directly related to the relative proportions of Hex A and Hex B isoenzymes [12]. The presence of the α subunit in Hex A means that its activation energy is significantly lower ($p<0.001$) than that of the Hex B [12]. The Hex isoenzymes were determined in seminal plasma using the apparent enzyme activation energy, and the results obtained in normozoospermic, oligozoospermic, azoospermic and vasectomized men are presented.

2. Materials and methods

Semen samples were obtained by masturbation after a 4-day period of sexual abstinence from 86 men with a mean age of 34.9±0.7 years (range 20–50 years). The total number of seminal plasmas studied were distributed into four groups: (I) normozoospermic (n=38); (II) oligozoospermic (n=17); (III) azoospermic (n=9); and (IV) vasectomised which were actually azoospermic (n=22). The spermatozoa count, sperm motility and vitality assessments were performed after liquefaction within 1 h of ejaculation according to Wolrd Health Organization (WHO) standard methods [13]. A portion of unprocessed semen was centrifuged and the supernatant free from spermatozoa was stored at −37 °C to determine the Hex activity. In accordance with WHO criteria, it was considered that normozoospermic ejaculates had >20 million spermatozoa/ml and oligozoospermic ejaculates had <20 million spermatozoa/ml [13].

The enzymatic activities of Hex were determined using 3,3′-diclorophenolsulphoftaleinil *N*-acetyl-β-D-glucosaminide as a substrate, with reagents commercialised by Shionogi and Co. (Osaka, Japan) in a Cobas Bio centrifugal analyzer (Roche Diagnostics) programmed as follows: units U/l; calculation factor 863; standard 1–3 conc. 0; limit 0; temperature (°C) 37.0; type of analysis 2; wavelength (nm) 575; sample volume (μl) 10; diluent volume (μl) 50; reagent volume (μl) 150; incubation time (s) 0; start reagent volume (μl) 0; time of first reading (s) 300; time interval (s) 30; numbers of reading 10; blanking mode 1; print out mode 2. To prepare the reagent solution, 8.2 ml of distilled water was added to each bottle containing lyophilized substrate and buffer. The possible inhibitory effect of the albumin on the Hex activity [14] was not considered, taking into account the low albumin concentration in seminal plasma samples (0.68±0.03 g/dl). The determination of enzyme activities was made in duplicate at 25, 30, 35 and 37° C, and the slopes of the Arrhenius plots and the apparent activation energies were calculated as described by Rej and Vanderlinde [15]. The heat inactivation of Hex A isoenzyme was carried out as previously described for blood plasmas [14]. In brief, seminal plasma samples adjusted to pH≈5.8 were incubated at 52 °C in closed Eppendorf® cups without air space above the samples, to avoid CO_2 loss and an increase in pH [16].

Statistical analysis of the data was carried out using the SPSS package (SPSS Inc., Chicago, USA). The Kolmogorov-Smirnov test was applied to check for normality. Non-parametric tests (Mann–Whitney *U*-test and Spearman's correlation coefficient) were used, as the data did not have any Gaussian distributions. Lineal regression analysis was performed using the Passing-Bablock method. Statistical significance was accepted as a p-value of less

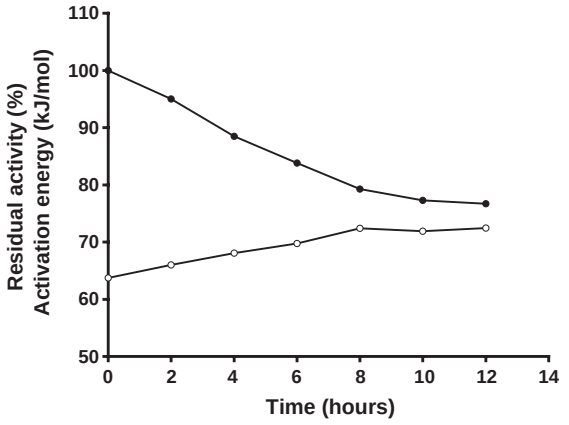

Fig. 1. Total Hex residual activity (●) and apparent activation energy (○) in a pool of seminal plasmas adjusted at pH≈5.8 and incubated at 52 °C.