N - ACETYL - B - GLUCOSAMINIDASE AND ITS ISOENZYME IN DIABETES MELLITUS

ESSAY

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BY

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N-acetyl ß-D-glucosaminidase (NAG), is a renal lysosomal enzyme located in proximal tubular cells of the kidney. ***(Kunin et al 1978).*** The hydrolytic enzyme NAG catalyses the cleavage of the hexosamine containing glycolipids, glycoprotein and mucopolysaccharrides that are abundant in cell membranes and other biological systems. ***(Orlacchio et al., 1984).***

The level of NAG have been reported to be raised in the serum of patients with diabetes mellitus ***(Orlacchio et al 1984).*** Increased NAG Activity in serum possibly is due to either increase in the rate of release or decrease in the rate of destruction of the enzyme. Increased production of lysosomal hydrolases followed by increased release into the circulation could also account for the increased NAG activity in the serum of diabetics. ***(Severini et al 1988).***

Two major isoenzymes, [A] and [B], in the serum of normal and diabetic patients are easily resolved, the concentration of isoenzyme [A] always exceeds that of isoenzyme [B]. Isoenzyme [B] concentration is lower in diabetics than in normals. An intermediate form [I] is more evident in diabetics with vascular activity. Thus measuring the isoenzyme forms may be a more sensitive indicator in diabetic status and its vascular complications. ***(Severini et al 1988).***

Urinary NAG has been shown to be a sensitive indicator of blood sugar control in insulin dependant diabetics. ***(Brouhard et al., 1984)***. Also a relationship was found between urinary NAG level and severity of vascular alteration in diabetes, in fact the greatest NAG activity was found in patients with kidney affection, therefore urinary NAG level may reveal the existence of a vascular nephropathy that is still too slight to be identified by the standard non invasive clinical examination. ***(Clemenzia et al 1987).***

This essay will try to review the serum and urinary NAG and its isoenzymes activity in diabetic patients. A detailed review of the different methods for NAG determination will be assessed.

N - ACETYL - ß - D - GLUCOSAMINIDASE

N-ACETYL-ß-D- GLUCOSAMINIDASE

N-acetyl-ß-D-glucosaminidase (2-acetamido-2-deoxy-ß-D-glucoside-acetamido-deoxyglucohydrolase, Ec 3.2.1.30) (NAG), a lysosomal hydrolase has been deteted in a wide variety of biological materials and in multiple molecular forms. NAG is particularly abundant in organs where high rates of glycoprotein, mucopoly saccharide and glycolipid turnover might be expected. ***(Orlacchio et al., 1986).***

The ubiquitous hydrolytic enzyme NAG catalyses the cleavage of hexosamine-containing glycoproteins and glycolipids that are abundant in cell membranes and other biological systems ***(Orlacchio et al., 1984).***

NAG activity is present in human tissues such as spleen, brain, liver and kidney (Table 1), it is also present in human serum and urine ***(Verpoorte, 1974).***

The chemical composition of NAG is represented in (Table 2).

Serum and plasma prepared from freshly drawn blood have the same NAG activity. Neither citrate nor EDTA has any effect, but storage at 2°c and repeated freezing and thawing seem to reduce the activity. Maximum activity is observed at pH 4.5-5. Hg^{2+} and Ag^{+} strongly inhibit the enzyme, and this inhibition is completely prevented by cysteine. Fe^{2+} also inhibits the enzyme in citrate buffer (pH 4.5), but not in succinate or acetate buffers of the same pH ***(Verpoorte, 1974).***

(Table 1)

Tissue	n	Mean Specific activity (and SD), U/g of protein (37 °C)	% inactivated at 56 °C, 15 min, mean (and SD)
Heart	4	11 (2)	31 (10)
Liver	5	48 (20)	35 (7)
Brain	4	15 (8)	21 (6)
Spleen	3	24 (9)	36 (11)
Muscle	4	10 (4)	58 (15)
Lung	4	43 (31)	36 (10)
Kidney	5	69 (19)	32 (18)
Ovary	1	9	32 (18)

Enzymatic activity of NAG in some human tissues

(Verpoorte, 1974)

(Table 2)

	Mol %
Amino Acids	
Lysine	9.0
Histidine	2.3
Arginie	3.5
Ammonia	10.3
Aspartic acid	9.5
Threonine	6.3
Serine	6.7
Glutamic acid	13.9
Proline	4.9
Glycine	5.2
Alanine	7.7
Half-cystine	2.1
Valine	6.3
Methionine	1.1
Isoeucine	2.4
Leucine	10.1
Tyrosine	2.8
Phenylalanine	4.4
Tryptophan	1.8
Carbohydrate	
Sialic acid	1.4
Neutral carbohydrate	30.0
Glucosamine	3.5
Galactosamine	Not dectable

(Chemical composition of NAG)

(Powell et al., 1983)

NAG isoenzymes:-

Tissue homogenates have two major forms, the **A** (acidic) and **B** (basic) form. ***(Price and Dance, 1972).***

The NAG activity in urine appears to exist mainly as the [A] and to a lesser extent the [B] isoenzymes component with only trace of intermediate forms [I_1] and [I_2] components ***(Kind, 1982).***

In normal serum the NAG activity can be resolved into at least four components, the major part of the activity is the [**A**] form, the [**B**] form only present in trace amounts in freshly drawn plasma. Two intermediate forms [I_1] and [I_2] are also present in serum and their concentration increases in storage. ***(Price and Dance, 1972).*** An additional isoenzyme [A^s] form is also present in urine samples containing blood ***(Nicot et al., 1987).*** [A^s] form is present also in serum and cerebral spinal fluid. The [A] component of serum can be distinguished from the [A] component of liver by ion-exchange chromatography, gel filtration, whereas the [A] component of urine could not be distinguished from the [A] component of liver by these procedures. ***(Ellis et al., 1975).***

All NAG isoenzymes have similar Km Value or Michealis constant (The substrate concentration that produces half maximal velocity) and are similar in pH optima and in their response to enzyme inhibition. ***(Orlacchio et al., 1986).***

Heating at pH 4.4 and 50 °c destroys the activity of the acid form [A], whereas the basic form [B] remains stable. The intermediate forms [I_1] and [I_2] have a similar resistance to heat like [B] form under conditions in which the [A] component is rapidly denatured. ***(Verpoorte et al., 1974).***

It should be recognized that A:B ratio are not necessarily absolute figures but represent only the state of affairs at the conclusion of the assay procedure. The conversion of [A] to [I_1], [I_2] and [B] forms which occurs on storage is accelerated by heat treatment ***(Price and Dance, 1972).***

NAG is mainly present within the lysosomes near the luminal border of the renal tubular cells, but it may also be present in the [B] form on the ribosomes where the protein-enzyme molecule is synthesized before it is converted to the [A] form by sialylation. The enzyme may be present also in the intermediate forms in the Golgi system before it is fully packaged into the lysosomes in the [A] form, and may represent stages in sialylation. ***(Kind, 1982).***

The normal urinary excretion of NAG and its isoenzymes

The urinary excretion of NAG is subject to very little diurnal variation under normal conditions ***(Price et al., 1970).*** Circadian rhythm in the urinary excretion of NAG is documented. The acrophase (timing of highest point in a rhythm) of the circadian rhythm in urinary NAG activity occurs at 9:00 o' clock with 95 % confidence limits between 8:00 and 12:00 O'clock, and is similar to the timing of the circadian rhythm in urinary free cortisol. There is also sex difference in mesor (mean value of a rhythm) of the circadian rhythm in urinary NAG activity, with female subjects having a higher mesor and amplitude than male subjects. ***(Lakatue et al., 1982).***

The urinary concentration of enzyme depends on the rate of urine flow as well as the output of the enzyme by the kidney. If it is assumed that creatinine excretion is constant, it is possible to eliminate the effect of changes in the rate of urine flow by expressing the enzyme output per mg creatinine. In this way the output of enzyme from the kidney can be determined on a single urine sample without reference to timed collections ***(Price et al., 1970).*** The normal range of NAG excretion according to ***Maruhn et al., 1976*** is as follows :-

Females : 0.27 - 1.18 u/m mol creatinine.

median : 0.57 u/m mol creatinine.

Males : 0.19 - 1.06 u/m mol creatinine.

median : 0.46 u/m mol creatinine.

Severini et al., 1988 reported the total NAG activity in urine of normal subjects to be 88 ± 35 u/mg creatinine (mean ± SD), the percentage of urine isoenzyme [A] comprised 89.8 ± 4.0 % , [B] 6.9 ± 1.4 % while isoenzyme [I] 1.8 ± 0.8 %.

Gibey et al, 1984 reported that the NAG activity in normal urine is primarily represented by the [A] form isoenzyme, the ratio of A : B + I being greater than 7.

The normal NAG and its isoenzymes activity in serum :-

Woolen and Turner, 1965 reported that plasma NAG activity in normal subjects is different in males and, females and differ also in subjects below 40 years and those above 40 years.

- Males below 40 years it ranges from 0.44 - 1.03 µ mole/min/l with a mean of 0.78 ± 0.14 µ mole/min/l.

- Males above 40 years it ranges from 0.49 - 1.76 µ mole/min/l with a mean of 1.01 ± 0.24 µ mole /min/l.

- Females below 40 years it ranges from 0.51 - 1.02 µ mole/min/l with a mean 0.73 ± 0.12 µ mole /min/l.

- Females above 40 years it ranges from 0.55 - 1.42 µ mole/min/l with a mean of 0.93 ± 0.18 µ mole/min/l.

Severini et al, 1988 reported that in serum the [A] component represents 71 ± 3.7 %, the [B] component 15.0 ± 2.1 %,and the [I] component 10.5 ± 2.9 % .