Effect of Different Compounds on 1-Aspartamido- β -*N*-acetylglucosamine Amidohydrolase from Human Liver

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The effect of various compounds on 1-aspartamido- β -*N*-acetylglucosamine amidohydrolase (aspartylglucosylaminase, EC 3.5.1.26) was studied. *N*-Acetylcysteine inhibited the enzyme non-competitively (K_i 3.2 mM), whereas 3-hydroxybutanone inhibited competitively (K_i 4.1 mM). Methionine, isoleucine and cystathionine apparently enhanced the enzyme activity. The enzyme had a mol.wt. of 63000 as determined by gel filtration. The present studies differentiate between the aspartylglucosylaminase from human liver and that obtained from various other sources.

Aspartylglucosaminuria, an inborn error of metabolism, is considered to be a disease of lysosomal origin. The disease was first described in two mentally retarded siblings in England (Jenner & Pollitt, 1967). By the end of 1976, about 100 cases had been verified in Finland (P. Aula, personal communication), two cases were reported in England (Jenner & Pollitt, 1967) and one in the U.S.A. (Isenberg & Sharp, 1975).

The enzyme from sheep epididymis involved in the splitting of the β -*N*-aspartylglucosylamine linkage was described by Murakami & Eylar (1965). Preliminary reports by Kaverzneva (1965) and Roston *et al.* (1965) indicate that aspartylglucosylaminase (1-aspartamido- β -*N*-acetylglucosamine aminohydrolase, EC 3.5.1.26) was also present in a mollusc (*Arion subfuscus*) and ram testis respectively. Most

of this enzyme activity is apparently located in the lysosomes (Mahadevan & Tappel, 1967; Oghushi & Yamashina, 1968). According to Makino *et al.* (1966), the aspartylglucosylaminase catalyses the reaction in Scheme 1, in which the second step is non-enzymic.

Kinetic studies have been done on enzyme from different sources with different degrees of purity (Clamp *et al.*, 1966; Eylar & Murakami, 1966; Makino *et al.*, 1966; Mahadevan & Tappel, 1967; Conchie & Strachan, 1969; Tarentino & Maley, 1969; Dugal & Strømme, 1977). The studies in the present paper are based on the following facts. (1) The activity of aspartylglucosaminase in aspartylglucosaminuria patients is very low (Pollitt & Jenner, 1969; Dugal, 1977). Any compound affecting the enzyme activity in a positive way may be of some use

Scheme 1. Action of aspartylglucosylaminase

from a clinical point of view. Such compounds (after further experiments) could be used to activate the enzyme and thus help eventually to control the incurable disease.

(2) A potent inhibitor of this enzyme may temporarily induce the disease and thus confirm the suggestion that aspartylglucosaminuria is the result of low aspartylglucosylaminase activity.

(3) This type of study may contribute to the knowledge of the specificity of aspartylglucosylaminase from human liver.

Materials and Methods

Materials were as described previously (Dugal, 1977; Dugal & Strømme, 1977). In addition *N*-acetyl-cysteine and 3-hydroxybutanone were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of the highest purity commercially obtainable. Enzyme assay was carried out as described by Dugal (1977).

Results

A review of the literature (Dugal & Dugal, 1978), showing that amino acids, their derivatives and many other compounds, including bivalent ions, have

inhibitory effects on the activity of amidase from different sources, led me to measure the effect of a series of compounds (Table 1) on the activity of the enzyme from human liver. Of a large number of amino acids and other compounds tested (see Table 1), only *N*-acetylcysteine and 3-hydroxybutanone had any inhibitory effect on enzyme activity, possibly as a result of the acetyl group common to these structures. The inhibitory effect of *N*-acetylcysteine was non-competitive, i.e. the $V_{\max}$ changed but the K_m value remained constant. The inhibitory effect of 3-hydroxybutanone was competitive, i.e. the $V_{\max}$ remained constant but the K_m value increased. Methionine, isoleucine and cystathionine apparently increased the enzyme activity.

Apparent K_i values of 4.1 mM and 3.2 mM were determined for 3-hydroxybutanone and *N*-acetylcysteine respectively.

The molecular weight of aspartylglucosylaminase, for partially purified enzyme fractions, has been shown by other authors to vary from 31 000 to 110 000 depending on the source. Mahadevan & Tappel (1967) report 31 000 mol.wt. for rat kidney enzyme, Yamashina (1972) reports 73 000 for hog kidney enzyme, and a mol.wt. of 110 000 has been reported for hen oviduct aspartylglucosylaminase by Tarentino

Table 1. Effect of various compounds on purified human liver aspartylglucosylaminase

The enzyme was preincubated for 30 min at 23°C with the compound (5 mM) and the activity was measured during the following 2 and 3 h of incubation in the presence of the compound. Assay was carried out in 0.32 ml at pH 7.7 as described by Dugal (1977). The value of the enzyme activity obtained without any compound after 2 and 3 h of incubation is 100%.

Compound	Activity (% of control)	Compound	Activity (% of control)
Glycine	92	Methylhistidine	100
Alanine	100	Phenylalanine	97
Serine	95	Proline	100
Homoserine	88	Hydroxyproline	95
Phosphoserine	100	Tyrosine	100
Taurine	100	Sarcosine	100
Cysteine	100	α -Aminobutyric acid	100
<i>N</i> -Acetylcysteine	33	γ -Aminobutyric acid	96
Cystathionine	120	β -Aminoisobutyric acid	111
Methionine	126	Glutamic acid	110
Methionine sulphone	88	α -Oxoglutarate	98
Methionine sulfoxide	100	<i>N</i> -Acetylglutamic acid	90
Leucine	110	Asparagine	98
Norleucine	95	Aspartic acid	100
Isoleucine	120	δ -Aminolaevulinic acid	93
Valine	100	Glucosaminic acid	100
Norvaline	99	Tosylarginine methyl ester	102
Threonine	92	Acetamide	90
Lysine	100	3-Hydroxybutanone	26
δ -Hydroxylysine	88	Acetylacetone	100
Arginine	106	Sodium acetate	100
Citrulline	100	<i>N</i> -Ethylmaleimide	100
Tryptophan	100	4-Hydroxy-3-methoxyphenethylamine	100
Histidine	97	3- <i>O</i> -Methyladrenaline	98

& Maley (1969). A column (1.6cm×60cm) of Sephadex G-200 equilibrated with 25 μ M-potassium phosphate buffer, pH 7.7, with a flow rate of 7.2 ml/h (each fraction 2.4 ml) was used for molecular-weight determination. The column was calibrated with proteins of different molecular weights: cytochrome c, 13000; chymotrypsinogen, 25700; ovalbumin, 43000; albumin, 68000; and immunoglobulin G, 156000. The aspartylglucosylaminase activity in 2.4 ml fractions was determined as described by Dugal (1977). A mol.wt. of 63000 was found for the human liver aspartylglucosylaminase.

Discussion

The enzyme responsible for formation of *N*-acetylglucosamine from aspartyl-*N*-acetylglucosamine has been included in the group of amidases (Murakami & Eylar, 1965; Makino *et al.*, 1966, 1968; Mahadevan & Tappel, 1967; Conchie & Strachan, 1969). Specificity studies (Makino *et al.*, 1968; Eylar & Murakami, 1966; Plummer *et al.*, 1968; Yamashina, 1972) of the enzyme suggest that the aspartyl moiety cannot be replaced by other amino acids, its presence being a prerequisite for the breakdown by this amidase of such a linkage. Further, the enzyme cannot split the amide linkage if the aspartic acid moiety is conjugated. The enzyme from human liver catalysing the formation of *N*-acetylglucosamine from aspartyl-*N*-acetylglucosamine is apparently also an amidase. Mahadevan & Tappel (1967) claim that amidase from rat kidney is inhibited by 3.2 mM-sodium acetate. On the contrary, I have found that human liver amidase is not affected by 5 mM-sodium acetate (Table 1). Aspartic acid and asparagine (both 1 mM) inhibited hog kidney amidase (Yamashina, 1972), but did not affect the amidase from human liver, even at 5 mM concentration (Table 1). In these respects human liver amidase apparently differs from that obtained from rat kidney (Mahadevan & Tappel, 1967) and hog kidney and serum (Yamashina, 1972). It has been reported that rat kidney amidase is inhibited by *p*-chloromercuribenzoate (Mahadevan & Tappel, 1967). I, however, could not find any effect of the thiol-group-specific *N*-ethylmaleimide or *p*-chloromercuribenzoate, even at 5 mM concentration. Glutathione and cysteine were also ineffective. In this way the human liver amidase also differs from that obtained from rat kidney or hog kidney. Our previous results (Dugal, 1977; Dugal & Strømme, 1977), showing that, other than Cu²⁺, bivalent ions were virtually ineffective at 5 mM concentration, also differentiate human liver enzyme from that obtained from rat kidney (Mahadevan & Tappel, 1967) and hog kidney and serum (Yamashina, 1972).

Hog kidney enzyme is inhibited by 50% at 1 mM-EDTA, whereas this concentration of EDTA has no

effect on the human liver aspartylglucosylaminase. This not only differentiates human liver enzyme from the hog kidney enzyme, but also indicates the metal-ion-independence of the human liver aspartylglucosylaminase.

My results (Dugal, 1977) indicated inhibition of enzyme activity by *N*-acetylcysteine, thus differentiating human liver amidase from amidase obtained from sheep epididymis (Murakami & Eylar, 1965), boar epididymis (Clamp *et al.*, 1966), pig serum (Makino *et al.*, 1966), human seminal fluid (Pollitt & Jenner, 1969), rat liver (Mahadevan & Tappel, 1967; Conchie & Strachan, 1969), the limpet *Patella vulgata* and rat epididymis (Conchie & Strachan, 1969), rat kidney (Mahadevan & Tappel, 1967), hen oviduct (Tarentino & Maley, 1969) and hog kidney (Yamashina, 1972).

Lineweaver-Burk plots carried out at various inhibitor concentrations indicated that *N*-acetylcysteine behaved as a non-competitive inhibitor (K_i 3.2 mM), whereas 3-hydroxybutanone inhibited competitively (K_i 4.1 mM). Cysteine had no effect on the enzyme activity, but *N*-acetylcysteine inhibited, suggesting that the inhibitory effect was probably due to the acetyl group.

Bearing in mind these arguments, the effect of other compounds such as acetamide, *N*-acetylglutamic acid, acetylacetone and 3-hydroxybutanone was studied. Only 3-hydroxybutanone inhibited the enzyme activity in a competitive fashion, resulting in a change in K_m whereas the V_{max} remained constant. In the hope of supporting these results, the search for any substance affecting the enzyme activity was extended to other compounds. Methionine apparently enhanced the enzyme activity, whereas methionine sulphone apparently decreased it and methionine sulfoxide had no effect (Table 1).

Hog kidney amidase was inhibited by 40 and 20% by aspartic acid and asparagine (both at 1 mM concentration) respectively (Yamashina, 1972), and hen oviduct enzyme was inhibited non-competitively by 5-diazo-4-oxonorvaline (modified norvaline) (Tarentino & Maley, 1969). These results, together with the specificity studies with aspartylglucosylaminase from different sources (Makino *et al.*, 1968; Clamp *et al.*, 1966; Plummer *et al.*, 1968; Tarentino & Maley, 1969; Yamashina, 1972), led me to search for other compounds influencing the amidase activity. Hoping to find similarities and/or differences between the enzyme from human liver and the amidase from other sources, I studied the effects of derivatives of different amino acids and various other compounds on this enzyme activity (Table 1). As summarized in Table 1, under my experimental conditions no compound other than *N*-acetylcysteine or 3-hydroxybutanone had any marked effect on the amidase from human liver. The molecular-weight determination gave a value of 63000, which is not

very different from that reported for hog kidney (Yamashina, 1972), but the effect of inhibitors (see above and Table 1) differentiates human liver aspartylglucosylaminase from that obtained from other sources.

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