GLUCOSE INHIBITION OF THE α-GLUCOSIDASE SPECIFIC FOR BASEMENT MEMBRANE AND COLLAGEN DISACCHARIDE UNITS

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1. Introduction

The enzyme 2-*O*-α-D-glucopyranosyl-*O*-β-D-galactopyranosyl-L-hydroxylysine glucohydrolase (EC 3.2.1.–) has been described in rat kidney [1], spleen [2] and chick embryo [3]. It appears specific for the hydroxylysine-linked disaccharide units, 2-*O*-α-D-glucopyranosyl-*O*-β-D-galactopyranosyl-L-hydroxylysine (Glc–Gal–Hyl) characteristic of collagens and basement membranes. It has been shown to be different from the α-glucosidase acting on neutral disaccharides such as maltose or *p*-nitrophenyl-α-D glucoside (EC 3.2.1.20) since it is inactive on these substrates [1–3]. Its substrate specificity seems to depend upon the presence of the positive charge of hydroxylysine [1]. The enzyme is able to bring about complete glucose removal from the disaccharide units of peptides as well as of whole basement membranes. It is believed to be involved in the catabolism of the basement membrane disaccharide units. In human [4] and experimental [5] diabetes, thickening of the glomerular basement membranes has been described: it is a characteristic alteration of diabetic microangiopathy. This thickening is accompanied by an increased content of disaccharide units [6–8]. A question raised therefore was that of the influence of the reaction products, especially glucose, on the activity of Glc–Gal–Hyl glucohydrolase in normal and diabetic kidney tissue. Any important effect of glucose could be relevant to the understanding of the basement membrane thickening in diabetes. In fact we show here a marked glucose inhibition of the enzyme activity which could contribute to the basement membrane thickening in diabetes. The kinetic parameters of glucose inhibition are compared with those of galactosylhydroxylysine, hexosamines and other inhibitors.

Abbreviations: Glc–Gal–Hyl, 2-*O*-α-D-glucopyranosyl-*O*-β-D-galactopyranosyl-L-hydroxylysine; [S], substrate concentration; [P], product concentration; [S] eq, [P] eq, concentrations at equilibrium; K_p, K_i, inhibition constants for product or effector; K_m, Michaelis constant; K_{eq}, equilibrium constant; V_1, V_2, maximal velocities of the reaction forming the product and the substrate, respectively; [Enz], enzyme concentration

2. Materials and methods

2.1. *Substrate and effectors*

Glc–Gal–Hyl was prepared from bovine kidney cortex basement membranes as in [1]. Glc–Gal–Hyl was radiolabeled by treatment with galactose oxidase followed by reduction with tritiated potassium borohydride as in [1] for Gal–Hyl. The specific activity obtained was 2.5 Ci/mol^{-1}. Gal–Hyl was prepared by mild acid hydrolysis of Glc–Gal–Hyl [1]. The various monosaccharides or derivatives and the aminoacids tested as effectors (table 1) were purchased from Sigma except for D-glucono-1-5-lactone purchased from Pfanstiehl (Waukegan IL).

2.2. *Enzyme preparation*

The 105 000 × *g* supernatants of rat kidney cortex homogenates were used for these studies since they are enriched in Glc–Gal–Hyl glucohydrolase and much more stable than the further purified fractions [1]. They were prepared from 7 month old normal or diabetic male Wistar rats, as in [1]. The diabetic rats had been injected i.v. with streptozotocin (Sigma) 55 mg/kg body wt, 23 weeks before sacrifice. Their plasma glucose level at sacrifice was 39 ± 2 (SE) mM, as measured by the glucose oxidase method; the

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normal rats had a plasma glucose level of 9 ± 0.2 mM in non-fasting condition ($p < 0.001$). The enzyme fractions were extensively dialyzed against 125 mM NaCl, 25 mM sodium phosphate buffer (pH 7.0) to remove all glucose. The protein content was measured according to [9].

2.3. *Standard assay for Glc–Gal–Hyl glucohydrolase activity*

Incubations with the dialyzed kidney enzyme and Glc–Gal–Hyl as substrate were performed for 1 h at 37°C and pH 4.4 as in [1]. Released glucose was measured by a micro-modification of the glucose oxidase procedure.

2.4. *Assay for Glc–Gal–Hyl glucohydrolase activity in the presence of glucose*

Glc–[^{3}H]Gal–Hyl (20 nmol, 2.5 Ci/mol) was dried with various amounts of unlabeled Glc–Gal–Hyl and of glucose in small glass tubes and then dissolved in 20 μl 1 M sodium phosphate buffer (pH 4.4). The dialyzed kidney enzyme (100 μl) was added and the mixture incubated for 1 h at 37°C. The reaction was terminated by the addition of 1 ml cold water followed by 0.5 ml 0.12 M $ZnSO_4$ and 0.5 ml 0.12 M $Ba(OH)_2$. After centrifugation 1.6 ml supernatant was applied to a Dowex 50 WX4 column (200–400 mesh), H^+ form (1 ml) which was eluted after extensive water washing with 1.5 M ammonium hydroxyde. This eluate was lyophilized, redissolved and deposited on Whatman 3 MM paper for high-voltage electrophoresis [10]. Electrophoresis was performed for 3 h under 1300 V with a pyridine acetate buffer 3.6 M (pH 3.5) followed by scanning with a Packard Radiochromatogram Scanner (fig.1). The [^{3}H]Gal–Hyl peak was measured and the total amount of Gal–Hyl released calculated after efficiency correction. The mean difference between duplicate results was 5.2%.

3. Results

Glc–Gal–Hyl glucohydrolase activity measured in the 105 000 × *g* supernatant of normal rat kidney cortex without any effector was 42.2 ± 2.0 (SE) nmol glucose released/h . mg protein; it was higher in the diabetic rat enzyme fraction: 66.4 ± 3.6 nmol/h . mg ($p < 0.001$).

Glucose exerted a marked inhibitory effect on Glc–Gal–Hyl glucohydrolase activity (fig.1,2). The effect was similar with the enzyme fractions from normal and diabetic rats when expressed in percentage inhibition. The double reciprocal plot was typical of competitive inhibition by a reaction product. A Dixon plot [11] confirmed the competitive type of inhibition and allowed the determination of K_p = 7.5 × 10^{-3} M (fig.2) [12]. Gal–Hyl also showed a competitive inhibitory effect on the Glc–Gal–Hyl glucohydrolase activity with $K_p = 4.0 \times 10^{-3}$ M (fig.3).

The inhibition of the enzyme by the reaction products was compared with the inhibition by glu-

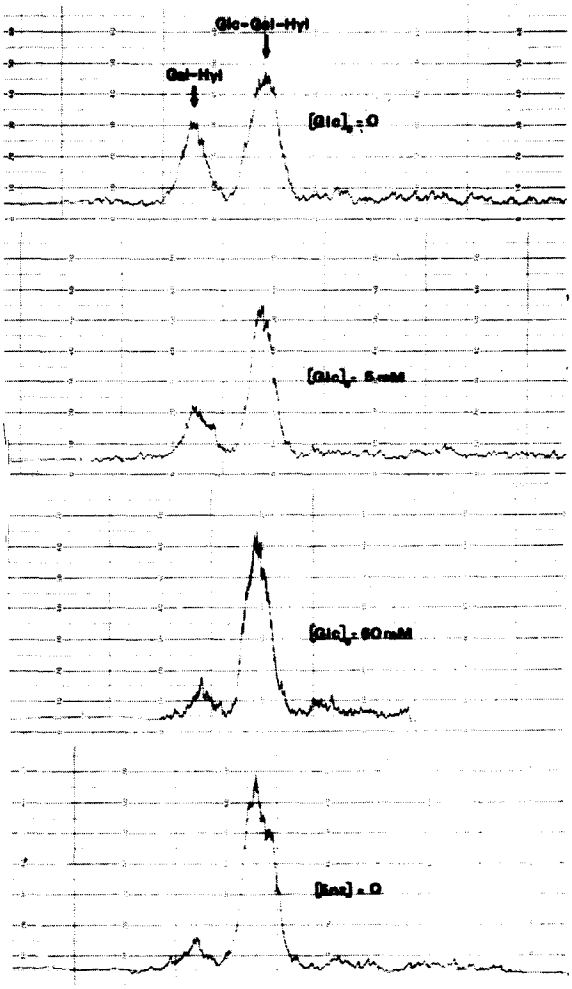

Fig.1. Glc–Gal–Hyl glucohydrolase assay in the presence of glucose. Determination of [^{3}H]Gal–Hyl released after incubation of Glc–[^{3}H]Gal–Hyl with the enzyme by high-voltage electrophoresis and scanning. Upper: incubation without glucose. Second and third scans: incubation with glucose 5 mM or 60 mM. Lower scan: control incubation without enzyme.

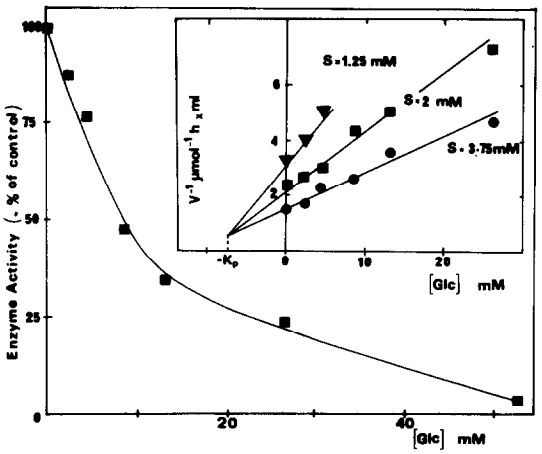

Fig.2. Glucose incubation of Glc–Gal–Hyl glucohydrolase from diabetic rat kidney cortex: (▼) [Glc–Gal–Hyl] = 1.25 mM; (■, inset and main curve) [Glc–Gal–Hyl] = 2 mM; (●) [Glc–Gal–Hyl] = 3.75 mM.

cosamine and mannosamine reported in [2] and related to the positive charge of the amine group next to the carbon 1 of the hexosamine molecule (table 1). Galactosamine was also found to be inhibitory but to a lesser degree. *N*-acetylhexosamines and *N*-acetylneuraminic acid were inactive as were galactose and hydroxylysine. D-Gluconolactone-1-5, a common inhibitor of the glucosidases, was active on the enzyme while D-galactolactone-1-4 was only slightly inhibitory.

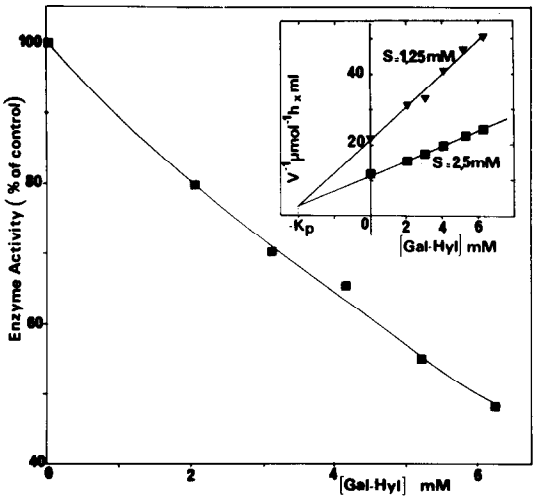

Fig.3. Gal–Hyl inhibition of Glc–Gal–Hyl glucohydrolase from normal rat kidney cortex: (▼) [Glc–Gal–Hyl] = 1.25 mM; (■, inset and main curve) [Glc–Gal–Hyl] = 2.5 mM.

Table 1
Effect of various monosaccharides or derivatives and of some basic amino acids on rat kidney Glc–Gal–Hyl glucohydrolase activity

Effector	Relative activity[a]	K_p or K_i (M)
None	100	
Glucose	73	7.5×10^{-3}
Galactose	99	
Mannose	102	
Glucosamine	26	7.0×10^{-4}
Galactosamine	69	5.0×10^{-3}
Mannosamine	0	1.2×10^{-5}
N-Acetylglucosamine	103	
N-Acetylgalactosamine	103	
N-Acetylmannosamine	107	
N-Acetylneuraminic acid	102	
D-Gluconolactone-1-5	15	n.d.
D-Galactolactone-1-4	86	n.d.
Gal–Hyl	65	4.0×10^{-3}
Hyl	104	
Lys	105	
Poly(L-Lysine)[b]	101	

[a] Final concentration of substrate was 2.5 mM and effector was 4.2 mM. The enzyme fraction used was a 105 000 × *g* supernatant from a normal rat kidney cortex homogenate dialyzed against 125 mM NaCl, 25 mM sodium phosphate buffer (pH 7.0)

[b] Concentration of lysine residues was 4.2 mM; mean M_r = 33 000 n.d., not determined

4. Discussion

Glucose concentration appears to be an important regulatory factor in the catabolism of collagen and basement membrane disaccharide units especially in the kidney. Renal cells, unlike adipocytes or muscle cells, appear to be freely permeable to glucose [13]. We have found the same glucose level in plasma (37.2 ± 1.4 mM) and in the kidney cortex homogenate (37.5 ± 2.8 mM) in diabetic rats ($p > 0.90$) (M. S., J. A. and Peyroux, unpublished). At the glucose concentration observed in the diabetic rats in this experiment, which is >4-times higher than in the normal rats, the percentage of enzyme inhibition is 87.5% with [S] = 2 mM (fig.2). In the kidney cortex, the concentration of disaccharide units is much lower than 2 mM and glucose inhibition must be even more important. Thus elevated glucose levels may contribute to the glomerular basement membrane thickening in diabetes by increasing the content in disaccharide units [6–8].

We have described and we confirm here a significant increase of Glc–Gal–Hyl glucohydrolase activity in the absence of glucose in the kidney cortex of diabetic rats with glomerular basement membrane thickening [14]. This increase of ~50% might be due to increased biosynthesis of the enzyme consecutive to the basement membrane thickening which represents an increase in the enzyme's substrate. However, increased glucose concentration in the kidney and glucose inhibition of Glc–Gal–Hyl glucohydrolase appear to be factors able to induce glomerular basement membrane thickening in diabetes.

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