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# SOME PROPERTIES OF HUMAN LIVER ACID α-GLUCOSIDASE

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## Summary

1. Albumin activates human liver acid α-glucosidase (α-D-glucoside hydrolase, EC 3.2.1.20). From the Arrhenius plot, pH-dependence and Lineweaver-Burk plots it can be concluded that this activation is not only due to stabilisation of the enzyme, but also influences the enzymatic activity. It is proposed that for optimal functioning human liver acid α-glucosidase needs a protein environment.

2. Glycogen has a competitive inhibitory effect on the hydrolysis of 4-methylumbelliferyl-α-D-glucopyranoside, in contrast to maltose which exhibits a non-competitive type of inhibition. It is concluded that two catalytic sites exist, one for glycogen and one for maltose, while both sites influence each other. With glycogen as substrate a break in the Arrhenius plot is found. This is not the case when maltose is used as substrate.

3. The effect of antibody raised against human liver acid α-glucosidase on the activity of human liver acid α-glucosidase is studied. No cross-reacting material could be demonstrated in the liver of a patient with glycogen storage disease Type II (M. Pompe, acid α-glucosidase deficiency).

## Introduction

Acid α-glucosidase (α-D-glucoside hydrolase, EC 3.2.1.20) has been isolated from various sources [1—6]. This enzyme degrades glycogen intralysosomally into glucose. Lack of this enzyme results in what is known as glycogen storage disease Type II (GSD Type II, M. Pompe) [7]. This deficiency of acid α-glucosidase leads to death in the first year of life, however, reports [8—12] have been made in which milder cases are presented. In these cases the muscular weakness is the most predominant clinical feature. Based on physicochemical and immunological properties of acid α-glucosidase from various human tissues no evidence is obtained for the existence of tissue-specific isoenzymes [6].

In the literature [4,13,14] attention has been paid to the kinetics of acid α-glucosidase. It is proposed [13,14] that the enzyme possesses at least two catalytically active substrate-binding sites. One site is specific for maltose and perhaps other oligosaccharides, and the other site is specific for polysaccharides like glycogen. These sites are in close proximity and interact with each other.

In the course of our study on the molecular basis of M. Pompe to find an explanation of the differences in the clinical manifestation of this disease, we isolated acid α-glucosidase from human liver. As far as we know only Belensky and Rosenfeld [15] reported some physicochemical properties of human liver acid α-glucosidase but no kinetics were presented. The purpose of this paper is to present some kinetic and immunological data of this enzyme.

## Materials and Methods

Acid α-glucosidase was isolated according to Auricchio et al. [1] with the modifications described earlier [6]. With this preparation antibodies were raised according to the method in ref. 6. Coupling of the antibody with CNBr-activated Sepharose 4B were performed according to the instructions of the manufacturer (Pharmacia, Uppsala, Sweden).

The enzymatic activities with the substrates 4-methylumbelliferyl-α-D-glucopyranoside (MUGlc), maltose and glycogen were performed according to the method in ref. 16.

Maltose was obtained from B.D.H., London, rabbit liver glycogen (AMP-free) from Boehringer (Mannheim) and human albumin from The Netherlands Red Cross Blood Transfusion Service.

## Results

Figs. 1A and 1B show the effect of the addition of albumin to the reaction medium, with glycogen and maltose as substrate. The addition of albumin results with both substrate (and also with MUGlc, not shown) in an increase of activity. This effect can be due to a protection of the enzyme for denaturation. However, if this is the case one should expect that as well with maltose as with glycogen as substrate equal increase of activity by albumin has to be found. With maltose as substrate an expected non-competitive activation by albumin is obtained, while with glycogen as substrate this non-competitive activation is not seen. The activation with maltose as substrate is about 40—50% of the activities without albumin of the various substrate concentrations. With glycogen this activation depends strongly on the substrate concentration used. At the lowest concentration (5 mg/ml) the activation is about 27% while at the highest substrate concentration (50 mg/ml) the value is about 330% of the activity without albumin. These data show that besides a stabilization of the enzyme by albumin, albumin has also another effect on the enzymatic activity, the expression of which seems to be dependent on the substrate used. It is even more remarkable that the activation by albumin is the largest at the highest glycogen concentration used, since we expected that glycogen might mask this phenomenon by stabilization of the enzyme by excess of substrate.

Further evidence for a more complicated effect of albumin is obtained

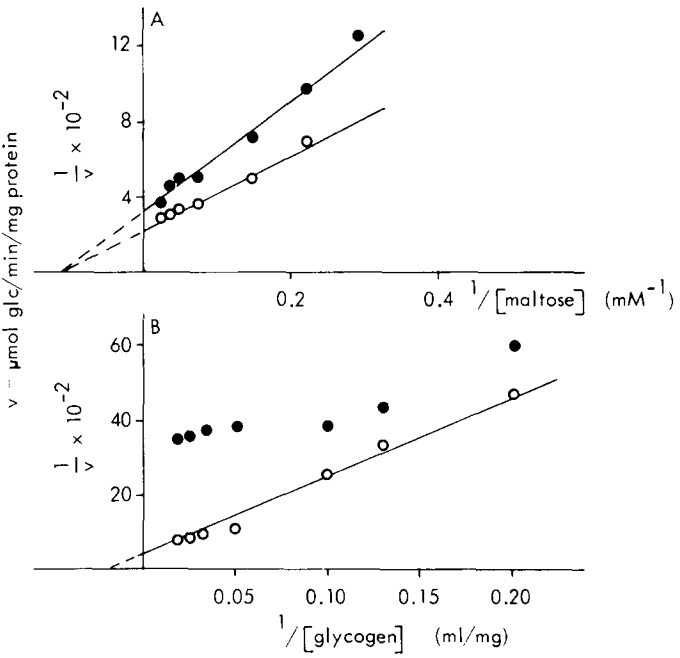


**Fig. 1. (A) The $1/v$ vs. 1/[maltose] plot of acid α-glucosidase in the absence (●———●) and presence (○———○) of albumin. Final concentration of albumin is 250 µg/ml. (B) The $1/v$ vs. 1/[glycogen] plot of acid α-glucosidase in the absence (●———●) and presence (○———○) of albumin. Final concentration of albumin is 250 µg/ml.**

from the Arrhenius plot (Fig. 2). With maltose (not shown) as substrate a straight line is obtained with an activation energy of 15 kcal/mol. With glycogen, however, a break point is seen at 21°C either in the presence or absence of albumin. The two slopes obtained allow the calculation of two energies of activation being 8.2 kcal/mol above 21°C and 12.7 kcal/mol below 21°C. The higher activation energy might reflect the higher viscosity of the substrate at the lower temperatures. This figure shows also that the line obtained with albumin present parallels the line obtained in its absence. If the activation by albumin were simply due to protection of the enzyme against denaturation, no parallel lines should be expected.

Fig. 3 shows the effect of pH on the activities of acid α-glucosidase with the substrates maltose and glycogen, respectively. With maltose as substrate the enzyme exhibits a much broader pH optimum than with glycogen. The pH optimum with glycogen depends on the presence of albumin. With the addition of albumin the pH optimum shifts from pH 5.0 to pH 4.5. Unexpectedly, the activation by albumin is much less in the alkaline region and the largest activation is seen in the acid region. This phenomenon pleads against a simple protection of the enzyme from denaturation by albumin. It is well known [5,6] for acid α-glucosidase that the enzyme is rapidly inactivated at higher pH values, but stable at acid pH values.

In Fig. 4 is shown that $1/v$ vs. 1/[MUGlc] plot in the presence of various amounts of maltose. In contrast to the literature [5] a non-competitive type of inhibition is found (Fig. 4A). For reasons that the intercepts on the abscissa

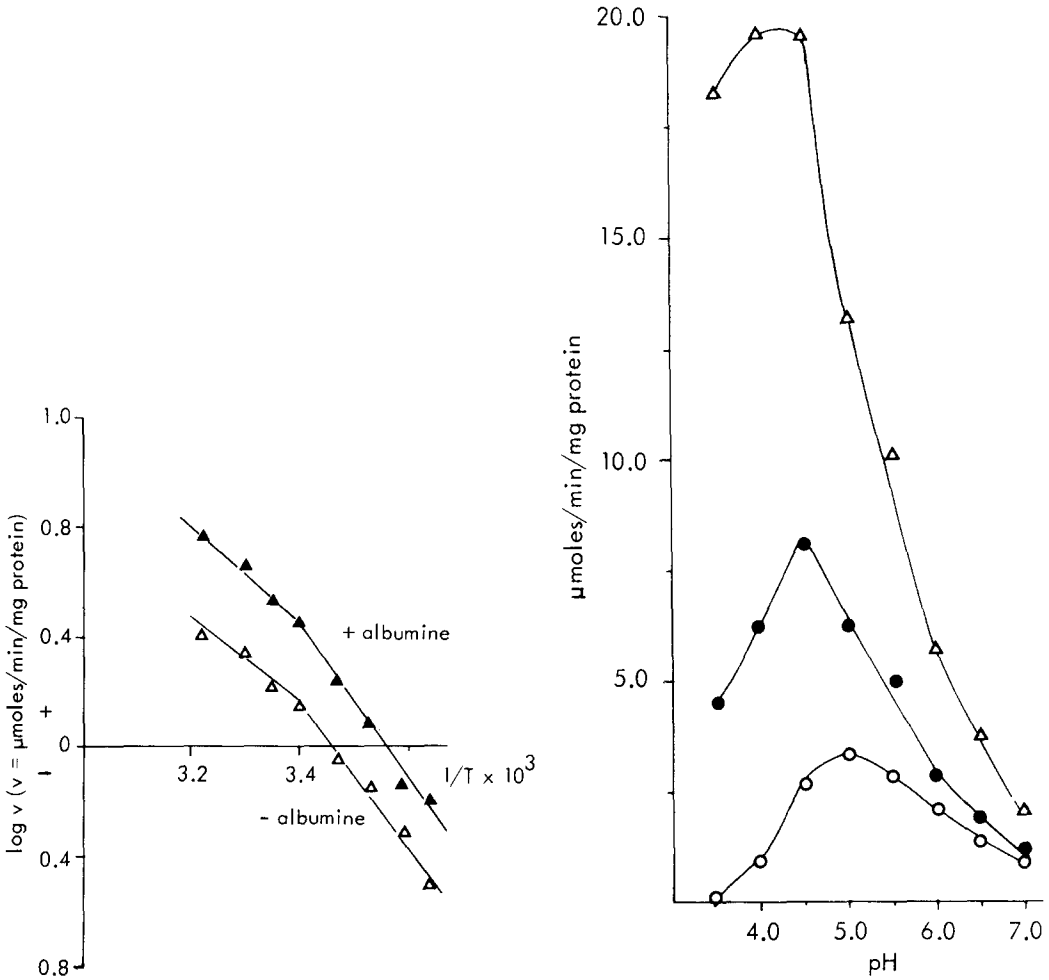


Fig. 2. Influence of albumin (830 μg/ml) on the log $v$ vs. $1/T$ of acid α-glucosidase. △———△, in the absence; ▲———▲, in the presence of albumin. The reaction is measured with glycogen as substrate (final concentration 20 mg/ml).

Fig. 3. The $v$ vs. pH plot of acid α-glucosidase with maltose and glycogen as substrate. △———△, maltose as substrate without albumin; ○———○, glycogen as substrate without albumin and ●———●, glycogen as substrate with albumin (830 μg/ml final concentration). Final concentration of maltose is 14 mM and for glycogen 20 mg/ml.

and ordinate are rather small, a Dixon plot was constructed (Fig. 4B). This plot shows clearly that maltose inhibits the hydrolysis of MUglucoside in a non-competitive way ($K_i$ = 25 mM). Fig. 5 shows the Dixon plot in which glycogen is the inhibitory agent in the reaction with MUglucoside. By extrapolation these lines do not intersect on the ordinate but in the second quadrant ($K_i$ = 45 mg/ml). This indicates that glycogen inhibits the hydrolysis of MUglucoside in a competitive way. It should be mentioned that the $K_m$ for glycogen (50 mg/ml) fits very well with the $K_i$ value (45 mg/ml). In contrast, this is not the case for maltose. The $K_m$ for maltose is 10 mM while the $K_i$ value is 25 mM.

Fig. 6 shows the activity vs. the enzyme concentration at various amounts

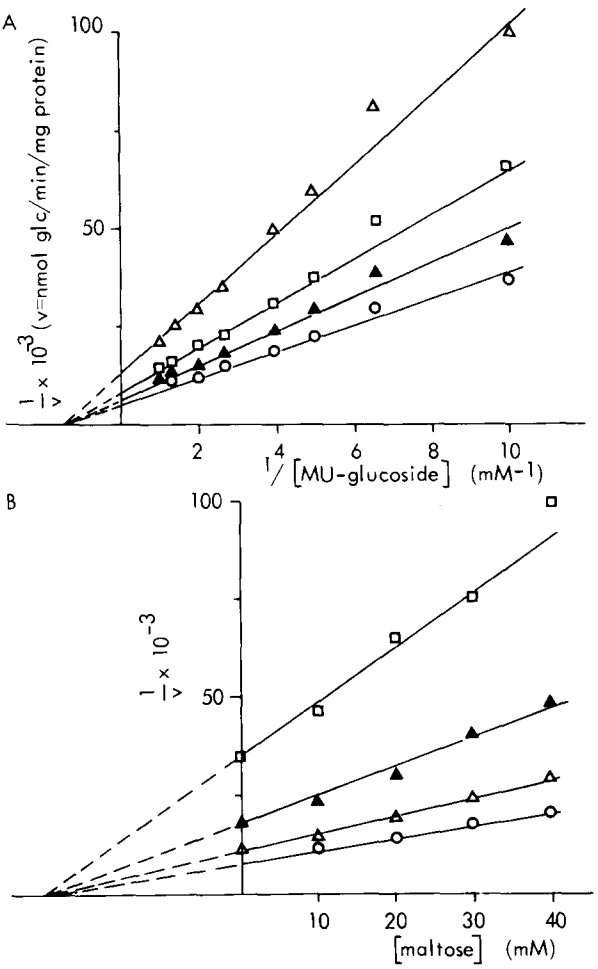


Fig. 4. (A) The $1/v$ vs. 1/[MU-α-glucoside] plot of acid α-glucosidase in the presence of various amounts of maltose: ○———○, control; ▲———▲, 10 mM maltose; □———□, 20 mM maltose and △———△, 40 mM maltose. (B) The $1/v$ vs. [maltose] plot of acid α-glucosidase at various concentrations of the substrate MU-α-glucoside. ○———○, 1.0 mM MU-α-glucoside; △———△, 0.5 mM MU-α-glucoside; ▲———▲, 0.25 mM MU-α-glucoside and □———□, 0.1 mM MU-α-glucoside.

of antibody raised against human liver acid α-glucosidase. The reaction has been measured with glycogen as substrate. This substrate is chosen because the antibody-antigen complex is inactive with glycogen in contrast to maltose as substrate. Up to the addition of 50 μl antibody the lines parallel the line in the absence of antibody. At higher concentrations of antibody the line deviates, which is also apparent by plotting the intercepts on the abscissa against the antibody concentration (see insert Fig. 6). Instead of a linear relationship the line curves downwards.

Neuwelt et al. [17] have developed an elegant method to detect whether cross-reacting material, partial or total identity of protein exists. This method implies the following procedure: antibody bound to Sepharose 4B is incubated with various amounts of material containing cross-reacting protein (blocking protein). The Sepharose 4B is settled down and the supernatant is sucked off

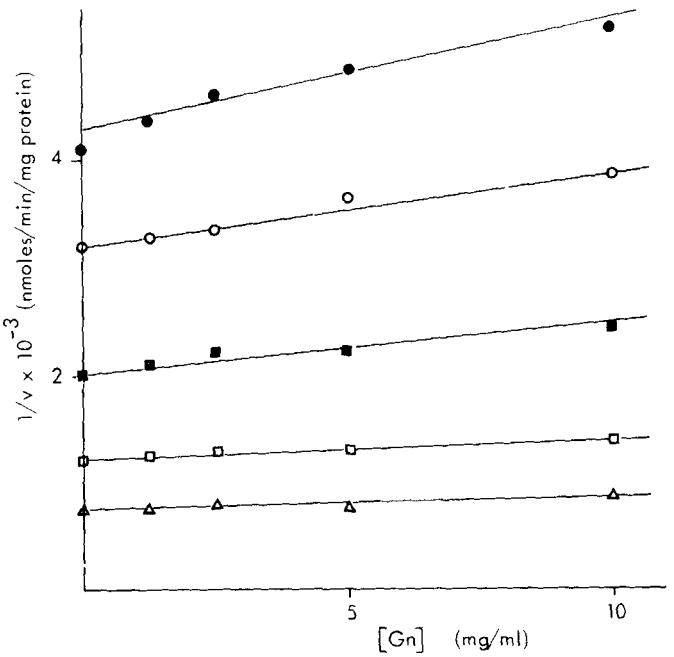


Fig. 5. The $1/v$ vs. [glycogen] at various concentrations of the substrate MU-α-glucoside. △———△, 1.0 mM MU-α-glucoside; □———□, 0.5 mM MU-α-glucoside; ■———■, 0.25 mM MU-α-glucoside; ○———○, 0.15 mM MU-α-glucoside and ●———●, 0.1 mM MU-α-glucoside.

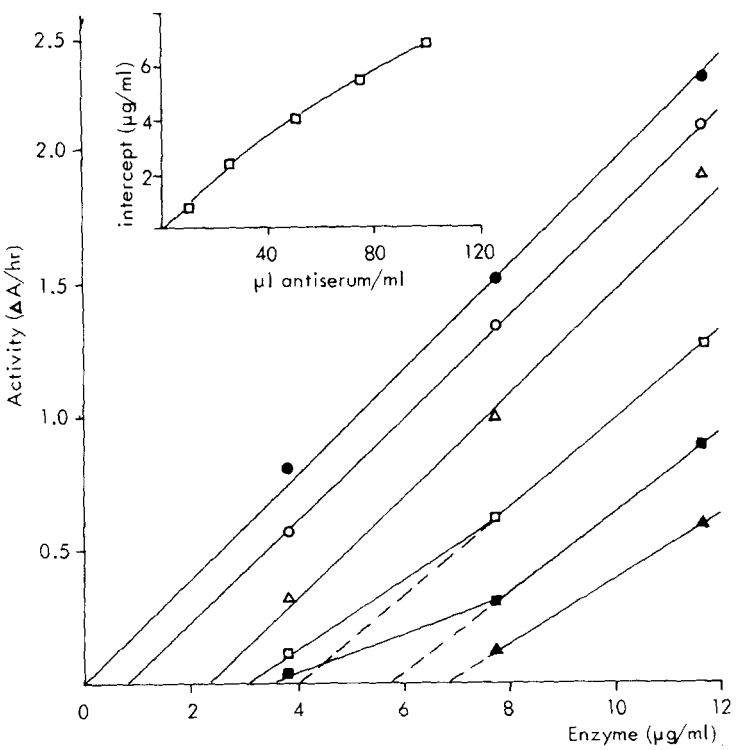


Fig. 6. The influence of antibody raised against acid α-glucosidase on the activity of acid α-glucosidase. Albumin is added (250 μg/ml). Substrate glycogen (20 mg/ml). ●———●, control; ○———○, 10 μl/ml antibody; △———△, 25 μl/ml antibody; □———□, 50 μl/ml antibody, ■———■, 75 μl/ml antibody and ▲———▲, 100 μl/ml antibody added.

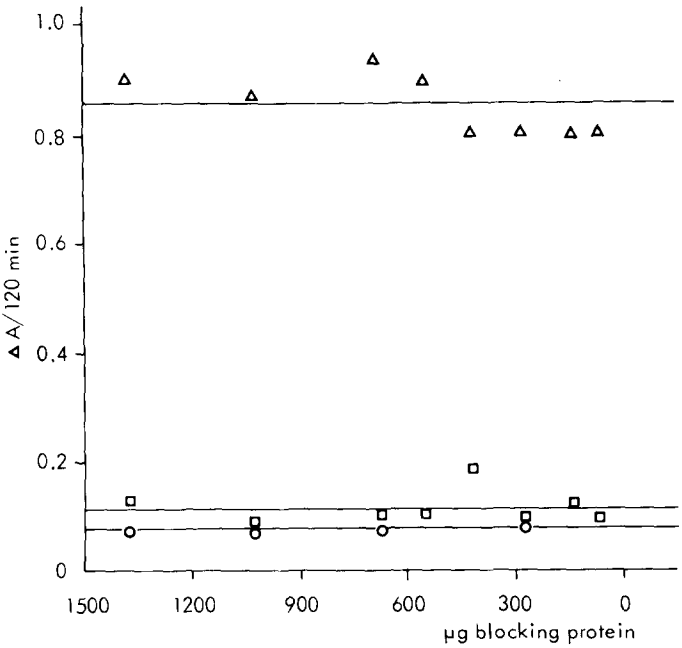


Fig. 7. The use of blocking primary enzyme immunoassay on acid α-glucosidase-deficient liver. The blocking protein is derived from patient with Type II glycogenosis. △———△, normal serum coupled to Sepharose 4B plus normal enzyme; ○———○, antibody coupled to Sepharose 4B plus normal enzyme; □———□, antibody coupled to Sepharose 4B plus various amounts of blocking protein. Amount of normal enzyme (liver homogenate), 610 μg protein; incubation temperature, 37°C; incubation time, respectively, 60 and 30 min; pH 7.4.

carefully. Then a fixed amount of enzymatic activity is added and incubated again. After incubation and sedimentation of the Sepharose 4B, the activity in the supernatant is measured. If increasing activity in the supernatant is found with increasing amount of blocking protein then cross-reacting material is present. If no activity can be detected in the supernatant then no cross-reacting material is present (the added amount of activity is bound to the Sepharose 4B). This method was applied to investigate if a liver, which possesses no acid α-glucosidase activity (from a patient suffering of GSD Type II), possesses cross-reacting material. For this reason the antibody is coupled to Sepharose 4B and the mutant liver was added at increasing amount with the addition of a fixed amount of acid α-glucosidase activity. The result is shown in Fig. 7. From this plot it can be concluded that the mutant liver does not contain cross-reacting material against the antibody raised to human liver acid α-glucosidase.

## Discussion

The enhancement of the activity by the addition of albumin cannot simply be explained by a stabilization of the enzyme. Besides stabilization, the presence of albumin has to influence the active center. If only stabilization of the enzyme occurred with the addition of albumin, one should expect at higher temperatures a larger activation than at low temperature. However, the Arrhe-

nius plot shows parallel lines in the presence and absence of albumin. Further evidence for a direct influence of albumin on the enzymatic activity is given in the pH vs. activity plot. The increase of the enzymatic activity by albumin can be due to a conformational change of the enzyme molecule.

From other experiments it is known that acid α-glucosidase is most stable at acid pH and inactivates at higher pH values. The addition of albumin, however, gives the largest activation at acid pH and much less at higher pH values. These data indicate that for an optimal functioning of acid α-glucosidase, especially with the natural substrate glycogen, a protein surrounding is necessary. An explanation on molecular basis for the break in the Arrhenius plot can be a change in the conformation of the enzyme, but it is also possible that the conformation of the polymer changes with the temperature. Such a break is not found with maltose as substrate (unpublished results), which might indicate a change of the substrate glycogen by changing the temperature.

The kinetic data presented give further support to the existence of two different catalytic sites. The inhibitory effects of maltose and glycogen on the hydrolysis of MUglucoside differ in that maltose exerts a non-competitive and glycogen a competitive nature of inhibition. This implicates that MU-glucoside combines at the same active site as glycogen. From the effect of maltose one can conclude that this disaccharide is bound on another active site and that both sites can influence each other. This is strengthened by the fact that $K_m$ value for maltose differs from $K_i$ value found. These conclusions are in agreement with those made for the active sites of rat liver [13] and rabbit liver [14] acid α-glucosidase.

The results presented in Fig. 6 are difficult to explain. A parallel set of lines has to be expected but a clear deviation is seen. This deviation is reflected in the non-linear relationship between the added amount of antibody and the amount of enzyme inactivated. In agreement with De Barsy et al. [5] and Brown et al. [18] but with a total different method no cross-reacting material was found in a liver of a child, who is deficient in acid α-glucosidase activity. De Barsy et al. [5] mentioned that the enzyme inactivated with alkaline pH (pH 10) has also lost its activity to combine with the antibody. From preliminary experiments we know that at this pH the enzyme is converted in different aggregational states. It is quite possible that the ability to combine with the antibody is lost due to this process.

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