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MALTASE, ISOMALTASE AND GLUCOAMYLASE ACTIVITIES OF THE ACID α-D-GLUCOSIDE GLUCOHYDROLASE ISOLATED FROM CATTLE LIVER

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SUMMARY

1. Purified preparations of cattle liver lysosomal acid α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) are able to debranch glycogen and completely hydrolyze the polysaccharide to glucose by acting as an exoglucoamylase. The enzyme also completely hydrolyzes β-amylase limit dextrin to glucose.

2. Glucose competitively inhibits the maltase activity of the α-glucosidase.

3. The relationship existing between the catalytic centers binding maltose, isomaltose and glycogen of the cattle liver acid α-D-glucosidase was studied by means of kinetic experiments.

As far as maltose and isomaltose are concerned, the present results suggest that the same center binds both substrates.

The relationship between the catalytic centers binding maltose and glycogen seems to be rather complex. The data suggest that, in addition to the single catalytic site which binds both maltose and glycogen, the polysaccharide may require additional binding sites on the enzyme molecule.

INTRODUCTION

Since the discovery by HERS[1] in 1963 of the existence of a lysosomal α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) in human tissues and the role played by this enzyme in the pathogenesis of the most severe form of glycogen storage disease, type II or Pompe's disease, numerous studies have been performed on the purification and mechanism of action of this acid α-glucosidase from a number of sources[2–13]. The enzyme has been purified to homogeneity from cattle liver[9] and extensively from rat liver[7,10] and has been shown to possess a broad specifity, being able to hydrolyze α-1,4- and α-1,6-glycosidic bonds in polysaccharides, glycogen and dextran, and disaccharides such as maltose and isomaltose[9,10,13]. Both enzyme preparations also showed transglucosidase activity, being able to incorporate glucose into glycogen[10,13].

In view of the broad specifity exhibited by the enzyme one wonders if the lysosomal glycogenolysis can be carried to completion by this enzyme only. The

existence of a lysosomal α-1,6-glucosidase has been postulated[14,15] but not yet found. JEFFREY *et al.*[11] have shown that the rat liver enzyme is able to completely degrade glycogen to glucose. On the other hand, rabbit liver enzyme did not completely degrade the polysaccharide[16].

In the present paper we have investigated the mechanism of action of cattle liver lysosomal α-glucosidase and shown that it is also able to completely degrade glycogen to glucose acting as an exoglucoamylase.

Kinetic experiments seem to indicate that maltose and isomaltose are hydrolyzed by the same active center and that the same active site is also shared by maltose and glycogen, but that the polysaccharide requires additional binding sites on the enzyme molecule.

MATERIAL AND METHODS

Enzyme

The cattle liver lysosomal α-glucosidase used throughout this study was purified to homogeneity as previously described[9].

Chemicals

Maltose and trehalose were obtained from Merck (Darmstadt, Germany). [^{14}C]-maltose (uniformly labeled, 5.7 and 7 mC/mmole) was purchased from the Radiochemical Centre (Amersham, England). α-Methyl glucoside, β-amylase and shellfish glycogen were from Sigma (St. Louis, Mo.). The polysaccharide was purified by dialysis against distilled water and by repeated precipitations with 50% ethanol (v/v). Isomaltose was from K and K Laboratories (Plainview, N.Y.), glucose from C. Erba (Milano, Italy). Maltose, isomaltose and glucose were found to be pure by thin-layer chromatographic analysis (see below). Turanose was purchased from British Drug Houses Ltd. (Poole, England).

Extent of hydrolysis of glycogen and β-amylase limit dextrins

The extent of glycogen hydrolysis was determined as follows. An incubation mixture of 1 ml contained 2 mg of glycogen, 57 μg of enzyme and 60 μmoles of sodium acetate buffer (pH 4.5). A small amount of toluene was added. The reaction was allowed to proceed at 37°. At various times (0–48 h) aliquots were withdrawn and used for determination of glucose by the glucose oxidase method[17], and total reducing power[18]. On the same aliquots thin-layer chromatography was performed in order to identify the products. When hydrolysis of β-amylase limit dextrins was followed, only the appearance of glucose was measured. Dextrins were prepared from glycogen according to the method of BAILEY AND WHELAN[19].

Enzyme assays

Maltase, isomaltase and glucoamylase activities were measured as described previously[9]. In the experiments involving glycogen inhibition of maltase activity, 1 ml of a mixture containing 1 μg of enzyme, 60 μmoles of acetate buffer (pH 4.5) and different concentrations of radioactive maltose was incubated at 37° for 60 min either alone (controls) or in the presence of glycogen (49 mg/ml). Aliquots of 0.1 ml were withdrawn after 0, 30, 45 and 60 min of incubation and the reaction was stopped

by heating at 100° for 2 min. Glycogen was then added to the controls to reach a final concentration of 49 mg/ml. Ethanol 50% (v/v) was used to precipitate the glycogen. This precipitation was required for good chromatographic separation of glucose from maltose when glycogen was present. When maltase activity was measured in the presence of glucose or isomaltose the procedure was identical except for the omission of ethanol precipitation.

Thin-layer chromatography

50 μl aliquots of each sample were applied on silica gel F 254 plates (E. Merck). The chromatograms were developed in butanol–ethanol–water (50:30:20, by vol.) and dried at room temperature. The chromatograms were sprayed with a mixture of equal volumes of 0.1 M $AgNO_3$ and 5 M ammonia and heated at 70° for 5 min. The gel corresponding to the spots due to glucose was scraped and suspended in 0.2 ml of distilled water. The suspension was centrifuged and an aliquot of the supernatant solution was added to a scintillation flask containing 12 ml of liquiflor (Nuclear New England Corp.) diluted 1:25 (v/v) with toluene, 2 ml of absolute ethanol and 0.3 ml of NCS solubilizer (Amersham-Searle), and counted in a liquid scintillation spectrometer.

RESULTS

Product identification

In order to establish whether the cattle liver lysosomal α-glucosidase is able to split both 1,4- and 1,6-glycosidic bonds and whether the enzyme acts as an exo- or endoglucosidase, we have followed the hydrolysis of glycogen and β-amylase limit dextrins. Glycogen is completely hydrolyzed by the acid α-glucosidase after 48 h of incubation. At intervals samples were analyzed for the total reducing power, for glucose, and the products were separated by thin-layer chromatography. The glucose content was found to be identical when measured either by the method of glucose oxidase or that of total reducing power. Thin-layer chromatographic analysis at each time revealed only the presence of glucose. Also β-amylase limit dextrins were completely hydrolyzed to glucose in the presence of the enzyme. The rates of hydrolysis for both glycogen and β-amylase limit dextrin were very similar.

Salt stimulation

JEFFREY *et al.*[10] observed stimulation of the rat liver α-glucosidase by cations, the maltase activity being stimulated to a lesser degree than the glucoamylase activity. The cattle liver enzyme is also stimulated by KCl in a similar way. This salt decreases the K_m for maltose, the v_{max} being unmodified.

Inhibition studies

We have previously reported that glucose inhibits the hydrolysis of the 6-bromo-2-naphtyl-α-D-glucopyranoside by the cattle liver enzyme[13]. We have now investigated the effect of glucose on maltase activity. Glucose inhibits this activity in a competitive way (Table I).

The kinetics in the presence of two substrates or in the presence of α-methyl-glucoside and trehalose, inhibitors of the rabbit liver and human tissue acid α-

TABLE I

SOME KINETIC PARAMETERS OF MALTASE, ISOMALTASE AND GLUCOAMYLASE ACTIVITIES OF PURE CATTLE LIVER ACID α-GLUCOSIDASE

$K_p = K_m$ measured in the presence of the inhibitor. K_i is calculated from the expression: $K_i = \frac{i}{\frac{K_p}{K_m} - 1}$ where i is the concentration of the competitive inhibitor; K_p and K_m were graphycally obtained from Lineweaver–Burk plots.

Substrate	K_m *(M)*	*Competitive inhibitor*	*Inhibitor concentration (mM)*	K_p *(M)*	K_i *(M)*
Maltose	$1.0 \cdot 10^{-2}$	Glucose	4	$1.9 \cdot 10^{-2}$	$4.3 \cdot 10^{-3}$
Isomaltose	$1.4 \cdot 10^{-1}$	Turanose	2	$2.5 \cdot 10^{-1}$	$2.6 \cdot 10^{-3}$
Isomaltose	$1.6 \cdot 10^{-1}$	α-Methyl glucoside	100	$2.4 \cdot 10^{-1}$	$2.0 \cdot 10^{-1}$
Isomaltose	$1.6 \cdot 10^{-1}$	Trehalose	200	$2.7 \cdot 10^{-1}$	$2.9 \cdot 10^{-1}$
Maltose	$1.0 \cdot 10^{-2}$	Isomaltose	100	$2.0 \cdot 10^{-2}$	$1.0 \cdot 10^{-1}$
Maltose	$1.0 \cdot 10^{-2}$	Glycogen	49*	$2.2 \cdot 10^{-2}$	41*
Maltose	$1.0 \cdot 10^{-2}$	α-Methyl glucoside	300	$3.1 \cdot 10^{-2}$	$1.4 \cdot 10^{-1}$
Glycogen	32*	α-Methyl glucoside	30	90*	$1.7 \cdot 10^{-2}$
Maltose	$1.0 \cdot 10^{-2}$	Trehalose	300	$1.8 \cdot 10^{-2}$	$3.6 \cdot 10^{-1}$
Glycogen	33*	Trehalose	22	85*	$1.4 \cdot 10^{-2}$

* Values in mg/ml.

glucosidase[12,20] and turanose, inhibitor of rat liver[2,6,11], human kidney[6] and cattle liver[9] enzymes, have been studied. Also the results of this study are reported in Table I. The isomaltase activity is competitively inhibited by turanose, α-methyl glucoside and trehalose; isomaltose competitively inhibits maltase activity. Furthermore, the maltase activity of the acid α-glucosidase is competitively inhibited by shellfish glycogen.

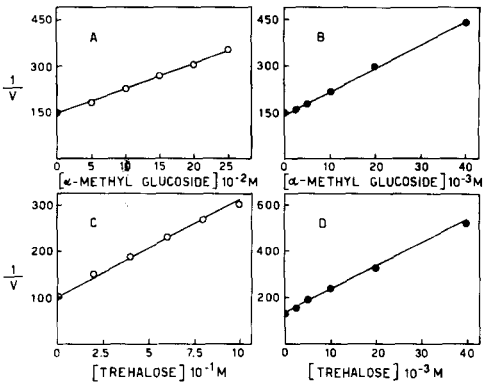

Fig. 1. Plot of the reciprocal of velocity of maltase (A and C) and glucoamylase (B and D) activities against different concentrations of α-methyl glucoside (A and B) and trehalose (C and D). Velocities are expressed as mmoles of maltose hydrolyzed (A and C) and mmoles of glucose produced (B and D) per min per mg of protein.

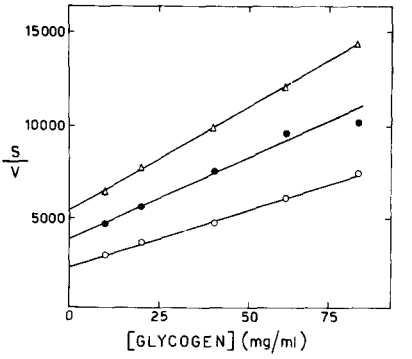

Fig. 2. Plot of substrate (mg glycogen/ml) to velocity (measured as mmoles of glucose produced per min per mg of protein) ratios of the acid α-glucosidase in the absence (○), and in the presence of 3 (●) and 6 mM (△) turanose.

α-Methyl glucoside and trehalose competitively inhibit both maltase and glucoamylase activities. When different concentrations of α-methyl glucoside and trehalose are used in the presence of a constant concentration of a substrate (maltose and glycogen), and the reciprocals of the velocity are plotted against various concentrations of the inhibitors, linear slopes are obtained (Fig. 1). This fact is consistent with the hypothesis that both inhibitors are bound to the active site(s) for maltose and glycogen. When, in fact, a competitive inhibitor combines with an enzyme at a site different from the substrate-binding site, hyperbolic instead of linear slopes are obtained.

Turanose, a competitive inhibitor of the maltase activity of cattle liver enzyme[9], is an inhibitor of the mixed type of the glucoamylase activity of the same protein (Fig. 2).

DISCUSSION

Since the acid α-glucosidase hydrolyzes not only α-1,4 (maltose), but also α-1,6-glycosidic bonds (isomaltose and dextran) the question arises as to whether the cattle liver enzyme is able to split the branching points of glycogen and to completely hydrolyze this polysaccharide. The results reported in the present paper show that the cattle liver acid α-glucosidase is able to completely hydrolyze polysaccharides like glycogen and β-amylase limit dextrin and therefore must be able to split the branching points of the glycogen molecule. This fact strongly supports the possibility that the lysosomal glycogenolysis is due to the action of a single enzyme.

The acid α-glucosidase could act on glycogen either as an exoglucosidase with a stepwise release of glucose as the only product of the reaction, or as an endoglucosidase with the intermediate production of oligosaccharides in addition to glucose. Since the total number of reducing groups is always equal to the number that can be accounted for as glucose and since no oligosaccharides are detectable as products of the enzymic action on glycogen, we conclude that glucose is the only product of this hydrolysis and, therefore, that the acid α-glucosidase is an exoglucoamylase.

Since the glucose inhibition of the acid α-glucosidase is of the competitive type, the site binding glucose is either related to or identical with the site binding maltose. Furthermore, since the K_i value is in the range of the glucose concentration of biological fluids, it is possible that glucose can play a regulatory effect on the enzyme activity *in vivo*.

In the present paper, kinetic experiments on the isomaltase and maltase activities in the presence of α-methylglucoside, trehalose and turanose suggest that the same active center is involved in the binding of maltose and isomaltose. In fact the K_i values for each inhibitor are similar when measured against maltose or isomaltose (Table I). If the cattle liver enzyme has only one active center for the hydrolysis of maltose and isomaltose, one would expect that one substrate would competitively inhibit the hydrolysis of the other and that the degree of inhibition would be determined by the relative affinities of the substrates for the α-glucosidase. Our results show that maltase activity is, in fact, competitively inhibited by isomaltose. Furthermore, the K_i value is similar to the K_m for isomaltose. Therefore, we conclude that a single catalytic site binds both maltose and isomaltose.

Glycogen competitively inhibits the maltase activity of the α-glucosidase with

a K_i similar to the K_m for glycogen. This result suggests that a single catalytic site is involved in the case of the cattle liver enzyme in the binding of glycogen and maltose. This differs from results obtained by JEFFREY *et al.*[11] in similar experiments with the rat liver enzyme, whose maltase activity glycogen inhibits competitively but with a K_i eight times the K_m for glycogen. α-Methyl glucoside and trehalose inhibit the maltase and glucoamylase activities of the cattle liver enzyme in a competitive way, but with different K_i values. Turanose inhibits maltase activity competitively, but gives mixed-type inhibition of the glucoamylase activity. Taken together, these data suggest that the maltose and glycogen binding sites are similar, but not identical. It is possible that the active center for maltose is also involved in binding glycogen but that the polysaccharide requires some additional binding sites on the enzyme molecule.

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