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Isolation and Study of a Liver α-Amylase. Comparison with Glycogen Phosphorylase Activity

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An oligomaltosaccharide-forming amylase has been observed in mice liver crude homogenate. This enzyme has been isolated by binding to amylose. Some of its functional parameters have been studied and compared with those of glycogen phosphorylase, demonstrating that amylase activity is not due to a glycogen phosphorylase isoenzyme. It has been further observed that amylase needs Ca^{2+} of Mg^{+2} and Cl^- for its activity.

Several authors have postulated that α and γ-amylases can mediate in glycogen degradation (5, 9, 13). According to ROSENFELD (9) the metabolic role of amylase is not yet well known, although it probably contributes in a rapid liberation of glucose in some tissues; however, this is not only due to amylase action (9). But it is possible that glucose liberation is produced by a combinated action of α-amylase and a glucosidase. Although it is supposed that amylase action in some tissues is lisosomal, in others it could be not so and would be used by the cell in moments of great requirement for glycogen degradation (1).

The control mechanism of amylase activity can be regulated by several hormones; adrenalin and insuline increase amylase activity in serum and decrease it in liver (6). Perhaps, the variation of the intracellular Ca^{2+} concentration could be another regulating mechanism as it is known that an increase of Ca^{2+} produces amylase activation (10, 15). Furthermore, a calcium mechanism for amylase regulation in mollusks in anaerobic conditions has been postulated (1).

MCGEACHIN *et al.* (7) and RUTTER *et al.* (12) have demonstrated the existence of α-amylases in mammalian liver, and a role in hepatic glycogen metabolism has been postulated (6, 11). TSUJINO *et al.* (14) have found a glucose-forming amy-

lase in human liver and more recently, REMESAR *et al.* (8) have demonstrated that fasting increases liver α-amylase activity in rat liver.

The object of this paper has been the comparison of amylase and phosphorylase activity in liver, and the determination of some functional parameters of the amylase isolated by binding to amylose.

Materials and Methods

Albine adult mice were sacrificed and their livers were removed immediately, and homogenized in 50 mM Imidazole-HCl buffer containing 0.1 % Triton X-100 at pH 7.5 or 6.3. The homogenate was centrifuged at 2,000 rpm, and the pellet was discarded. The supernatant was incubated with 2.5 % amylose during 10 min at 36° C, and then centrifuged at 5,000 rpm during 10 min; the supernatant was kept for enzymatic determinations. The amylose pellet was washed five times in 50 mM Imidazole-HCl buffer pH 7.5 with the same volume as that of the homogenate. The amylose suspension was used for the enzymatic determinations and kinetic studies of the amylase.

Enzymatic determinations. Amylase was determined by the method described by CLARK (2), based on the measure of reductor molecules appeared as result of amylase activity using 3,5 dinitrosalycilic acid. Enzyme aliquots were incubated in 50 mM imidazole-HCl buffer, pH 7.5 containing 1 % of soluble starch, at 36° C for different times (usually 0, 10, 20 and 30 min). Amylase activity was quantified in nkatals using a control curve of glucose.

Maltase activity was determined by measurement of glucose formed by enzymatic action on maltose. The incubation medium contained 1 % maltose in 50 mM imidazole-HCl buffer, pH 7.5. The enzymatic action was stopped by boiling the samples for five min. Glucose was measured by Merkotest BLUTZUCKER (GOD-PAP) method.

Glycogen phosphorylase activity was measured by the method described by FISCHER and KREBS (3) using 50 mM imidazole buffer, pH 6.6, containing 1 % glycogen, 75 mM NaF, 50 mM glucose-1-phosphate and 5 mM AMP. Incubations were carried out at 36° C and the phosphate produced in the incubated samples for 0, 10, 20 and 30 min was estimated by the method of FISKE and SUBBAROW (4).

Results

Activity of the enzymes. Aliquots of liver crude homogenate and others of amylose suspension were incubated as indicated in methods for the determination of amylase, maltase and glycogen phosphorylase activities.

In liver crude homogenate amylase activity was detected in order of 100 nkat per ml. However, glucose formation from starch produced by these homogenates was very low (1 nkat/ml).

Maltase activity in the homogenate was also very low (2 nkat/ml).

Amylase activity in amylose suspension was 30 nkat/ml. However, in this suspension no maltase activity was found or glucose formation from starch.

This suggests that glucose is not the final product of amylase activity.

Glycogen phosphorylase activity was also detected in liver crude homogenate and amylose suspension and was used for comparative studies.

Influence of pH on amylase and phosphorylase activity. Aliquots of amylose suspension were incubated as indicated in methods, at several pH for determination of phosphorylase and amylase activities. Amylase presented two optimal pH at 6.3

and 7.5. However phosphorylase activity presents only one at pH 6.6.

Effect of incubation temperature on amylase and phosphorylase activities. Aliquots of amylose suspension were incubated at different temperatures at pH 6.3 and 7.5 for amylase and 6.6 for phosphorylase. These incubations were carried out between 0° C and 75° C. It has been observed that amylase has a maximal activity at 45° C, decreasing at higher temperatures and disappearing totally at 75° C; in the same grade at pH 6.3 or pH 7.5. However, phosphorylase activity has a maximum at 37° C, decreasing at higher temperatures.

Effect of different substances on amylase and phosphorylase activity. Amylase and phosphorylase were determined in presence of different molecules whose action on phosphorylase activity is well known. Incubations in presence of hydroxylamine, piridoxal phosphate, ATP, AMP, glucose-1-phosphate and uridin-diphosphate-glucose were carried out for this purpose. Figure 1 shows the action of hydroxylamine on amylase and phosphorylase activities; phosporylase activity decreases in a continuous way in presence of crescent concentrations of hydroxylamine. However, amylase activity does not change until a hydroxylamine concentration of 10^{-2} M, decreasing quickly at higher concentrations. Hydroxylamine is a piridoxal phosphate inhibitor and these results could demonstrate that amylase does not need the presence of piridoxal phosphate as a coenzyme, in opposition to what occurs with phosphorylase.

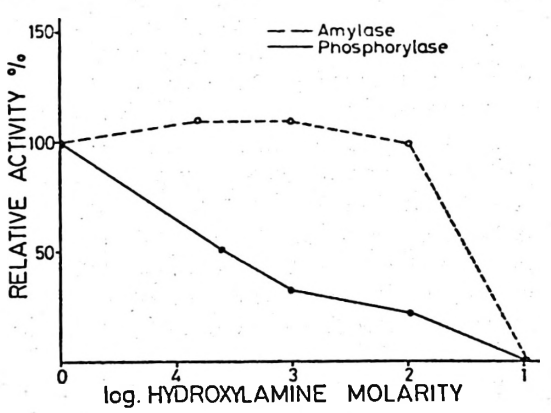

Fig. 1. *Effect of hydroxylamine on amylase and phosphorylase activities.*

On the other hand, the variations of amylase activity are not significant in presence of different concentrations (10^{-5} M to 10^{-2} M) of piridoxal phosphate, ATP, AMP, glucose-1-phosphate, glucose-6-phosphate und UDPG. These results demonstrate that none of these substances produce alosteric regulation on amylase activity.

Effect of EDTA on amylase activity. Amylose aliquots were incubated in presence of different concentrations of EDTA to determine amylase activity. It has been observed that amylase activity increases in presence of low concentrations of EDTA (2×10^{-4} M). However, activity decreases at higher concentrations, becoming null at an EDTA concentration of 10^{-2} M. Assays were carried out to estimate recuperation of amylase activity after 10 mM EDTA treatment. For this purpose 60 mM of Mg^{2+}, Ca^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} and both Ca^{2+} and Mg^{2+} together were used. Recuperation of amylase activity produced by Mg^{2+} was 114 %; Ca^{2+}, 86 %; Ca^{2+} and Mg^{2+}, 100 %, and Ba^{2+}, 46 %. The other ions did not produce recuperation of amylase activity. Figure 2 indicates the results of these assays.

Amylase activation by ions. Amylase activity was determined in presence of Ca^{2+}, Mg^{2+}, PO_4^{3-} and Cl^- at different concentrations. Figure 3 illustrates the results of these assays; Mg^{2+} and Ca^{2+} increase amylase activity stabilizing at 10^{-4} M concentration. However, PO_4^{3-}

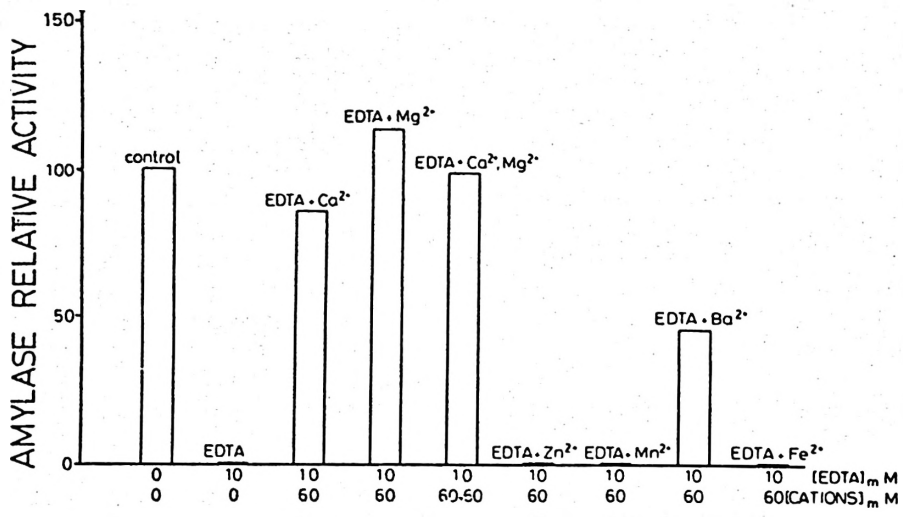

Fig. 2. *Effect of several divalent cations on amylase activity recuperation after 10^{-2} M EDTA treatment.*

does not produce at significant increase of amylase activity.

The effect of Cl^- on amylase activity was studied using Imidazole-acetic acid buffer at pH 7.5. Amylase activity was then determined at different Cl^- concentrations. Figure 3 illustrates that this enzyme needs Cl^- for its activity. This activity is practically null when Cl^- is absent and increases in function of the concentration of Cl^-, stabilizing at 10^{-3} M.

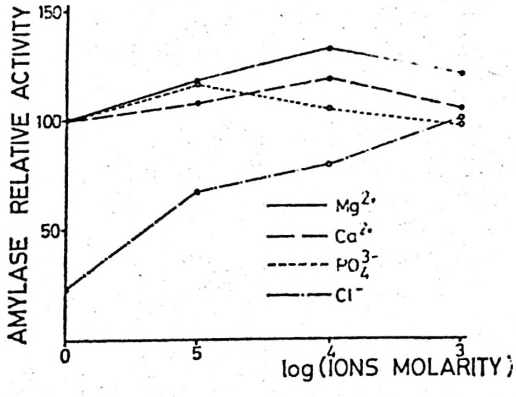

Fig. 3. *Effect of some ions concentration on amylase activity.*

Discussion

In mouse liver crude homogenate there has been found an amylase which hydrolyses starch giving oligosaccharides. However, using starch as substract, glucose forming by these homogenates is low. Maltase activity is also low. In amylose suspension there hasn't been found maltase activity; neither has there been found production of glucose using starch as substrate. Amylase binds to amylase while maltose stays in the homogenate. This supports that liver amylase activity gives oligomaltosaccharides which could be slightly hydrolysed to glucose by a glucosidase.

On the other hand, the study of amylase isolated on amylose demonstrates that some of its functional parameters are totally different from those of glycogen phosphorylase, also isolated on amylose in low quantities. Amylase optimal pH seems to demonstrate a non lisosomal localization of this enzyme.

It has been demonstrated that the effect of the temperature on amylase and glycogen phosphorylase activities is different too. Amylase does not need piridoxal

phosphate as coenzyme. On the other hand, it has been observed that non of phosphorylase alosteric regulators affect amylase activity, which confirms a non alosteric regulation of hydrolases.

It has been observed that the concentration of ion Cl^- could be the only regulating system of this enzyme. Ion Ca^{2+} or Mg^{2+} are also indispensable for amylase activity under the conditions studied. There must exist a strong union between the enzyme and one of these ions thus amylase activity only disappears in presence of EDTA. This could tentatively explain why amylase works even without Ca^{2+} or Mg^{2+} in the medium. However, it has not been observed amylase activity in a medium without Cl^- and it has been proved that this activity increases in function of the concentration of Cl^{-1}, stabilizing at 10^{-3} M.

Resumen

En homogenizados de hígado de ratón existe una α-amilasa que forma oligomaltosacáridos. Este enzima se aisló por fijación sobre amilosa. Se han estudiado algunos de sus parámetros funcionales, comparándolos con los de la glucógeno fosforilasa, demostrándose que la actividad amilásica no es debida a un isoenzima de la fosforilasa. Se ha observado que la amilasa necesita para su actividad Cl^- y Ca^2 ó Mg^2

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