EFFECT OF UREA ON ENZYMATIC ACTIVITY AND ELECTROPHORETIC MOBILITY OF ACID γ-AMYLASE (α-GLUCOSIDASE)

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Summary

Treatment of homogeneous preparation of acid γ-amylase, EC 3.2.1.3, (α-glucosidase) from rabbit liver by 3M urea leads to formation of two protein components, differing in electrophoretic mobility and substrate specificity. The slower-moving component split glycogen and did not split maltose; on the contrary, a faster-moving component of enzyme catalyzed the breakdown of maltose and was not able to hydrolyze glycogen.

In recent years several methods have been developed describing extensive purification of acid γ-amylase, EC 3.2.1.3, (α-glucosidase) from various mammalian organs (1-5).

All γ-amylase preparations regardless of the method used, possessed broad specificity, splitting, in particular, α-1,4-glucosidic bonds in the disaccharide maltose (maltase activity) and in polysaccharides glycogen and starch (glucoamylase activity).

Bruni et al. (6) demonstrated that homogeneous γ-amylase (α-glucosidase) from bovine liver with a molecular weight of about 107.000 dissociated in 5M guanidine-HCl solution into 4 inactive subunits with a similar molecular weight (25.000). The activity of the enzyme in polyacrylamide gel was determined by these authors using 6-bromo-2-naphthyl-α-D-glucopyranoside as substrate (2).

The present paper deals with the effect of urea on electrophoretic mobility and enzymatic activity of γ-amylase from rabbit liver. To study this effect, a method was developed for determination of both maltase and glucoamylase activities of γ-amylase in polyacrylamide gel before and after treatment by urea, using natural substrates of γ-amylase, maltose and glycogen. This method was previously used by Dahlqvist and Brun (7) for the histochemical demonstration of some disaccharidase activities.

Methods

γ-Amylase was purified as described previously (5) by means of adsorption of the enzyme on a Sephadex G-100 column, with subsequent desorption from dextran gel using the specific competitive inhibitor of γ-amylase, α-methylglucoside.

γ-Amylase activity was estimated by formation of glucose from maltose and glycogen. Glucose was determined by the glucose-oxidase method.

Electrophoresis of the enzyme was carried out in polyacrylamide gel at low pH as described by Welfle (8). The degree of polymerization of the gel was 7.5%. The gel was stained for proteins with 0.1% Coomassie Brilliant Blue in 7% acetic acid and destained with 7% acetic acid. Runs were performed at 5°C for 150 min. with 2.5 ma per tube. To demonstrate maltase activity of γ-amylase, glucose-oxidase was incorporated into the gel at the final concentration of 200 μg/ml. After electrophoresis the gels were incubated in the dark at 37°C in 0.4M phosphate buffer, pH 6.0, containing 10 mg/ml of maltose, 0.25 mg/ml of Nitro BT (2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)-ditetrazolium chloride) and 0.15 mg/ml of phenazine methosulfate. To demon-

strate glucoamylase activity of γ-amylase, glycogen, as well as glucose-oxidase, were incorporated into the polyacrylamide gel, the concentration of glycogen in the gel being 20 mg/ml. After electrophoresis the gels were incubated in 0.4M phosphate buffer, pH 6.0, containing 0.25 mg/ml of Nitro BT and 0.15 mg/ml of phenazine methosulfate. Incubation for 10-15 hrs was sufficient for the color to develop. Enzymatic reaction was stopped by immersing the gels into 5% trichloracetic acid solution. The proteins, undergoing denaturation in trichloracetic acid, were washed out by 6M urea solution, the colored product of the reaction remaining undissolved.

γ-Amylase, present in the gel, catalyzes either the hydrolysis of maltose diffusing from the incubation mixture into the gel (maltase activity) or the hydrolysis of glycogen previously incorporated into the gel (glucoamylase activity). In both cases the reaction leads to formation of glucose, which is oxidized by glucose-oxidase incorporated into the gel. The glucose liberated is demonstrated by means of a coupled enzymatic reaction yielding finally an insoluble violet-blue precipitate. Formation in a polyacrylamide gel column of a violet-blue band indicated the presence of γ-amylase activity.

Control experiments showed that in the electrophoretic system used γ-amylase migrated faster than glucose-oxidase and that the presence of glycogen and glucose-oxidase in the gel had no effect on electrophoretic mobility of γ-amylase.

Results and Discussion

The homogeneity of γ-amylase was tested by disk polyacrylamide gel electrophoresis. As can be seen from Fig.1 A, the enzyme migrated as one protein. Determination of maltase

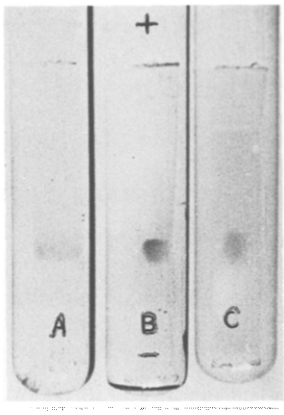

Fig.1. Disk electrophoresis of acid γ-amylase. The electrophoresis was performed on 7.5% polyacrylamide gels (2.5 mA per tube, 150 min., 5°C). A. Gel stained with Coomassie Brilliant Blue for protein. B. Gel stained for maltase activity. C. Gel stained for glucoamylase activity. About 30 μg of enzyme protein in 50 μl of 12.5 mM glycine-HCl buffer, pH 2.0 were applied to each gel. Experimental details described in the text.

activity of γ-amylase (Fig.1 B), as well as of glucoamylase activity (Fig.1 C), demonstrated that the bands, representing activities, coincide with the protein band. Treatment of γ-amylase by varying concentrations of urea revealed significant differences in the pattern of hydrolysis of maltose and glycogen. The glucoamylase activity of γ-amylase appeared to be much more sensitive to urea action than maltase activity (Fig.2). At 3M urea concentration no glycogen breakdown was observed, while maltase activity was lowered only twice under these conditions. At 6M urea concentration 20% maltase activity was still retained. Effect of urea was reversible and both activities of γ-amylase were fully restored on removing urea by dialysis or dilution.

Fig.3 presents data on electrophoresis of homogeneous γ-amylase in the presence of 3M urea. Treatment by urea results in formation of two components differing in electrophoretic mobility (Fig.3 A). Estimation of enzymatic activities of these

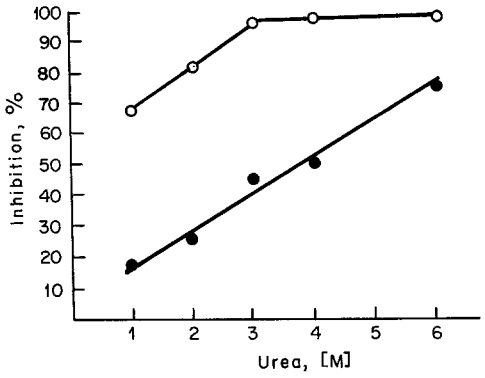

Fig.2. The effect of urea on maltase (•) and glucoamylase (○) activities of γ -amylase. The preincubation of the enzyme - 2 hrs at 37°C in 0.1M acetic buffer, pH 4.8 with various amounts of urea. Enzymatic reactions were performed also in the presence of urea. Glucose was estimated with glucose-oxidase after preliminary dilution.

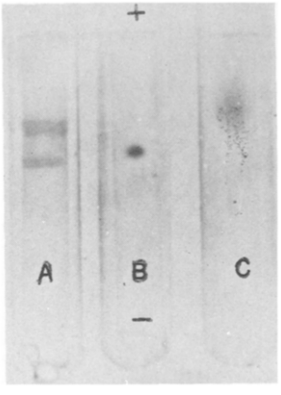

Fig.3. Disk electrophoresis of acid γ -amylase in the presence of 3M urea. The electrophoresis was performed on 7.5% polyacrylamide gels in the presence of 3M urea (2.5 mA per tube, 105 min., 5°C). Enzymatic reactions developed after removal of urea. A.B.C. - the same as in Fig.1.

components, carried out in polyacrylamide gel columns after removal of urea, showed that a slower-moving component possessed glucoamylase activity and was devoid of maltase activity (Fig.3 C). On the contrary, a faster-moving component split maltose and did not split glycogen (Fig.3 B). The electrophoresis of the enzyme in the presence of 6M urea gave four protein bands.

It was demonstrated earlier that there are significant differences in the pattern of hydrolysis of maltose and glycogen by γ-amylase. In particular, pH optimum of maltose breakdown is shifted to the acid zone as compared with that of glycogen breakdown (1,4,9). Hydrolysis of glycogen, in contrast to that of maltose, is inhibited competitively by α-methylglucoside and trehalose (10) and is activated more intensively by univalent cations (4), while NaF inhibits maltose breakdown, having no effect on glycogen breakdown (11).

Application in the present studies of a newly developed method of demonstration of γ-amylase activity in polyacrylamide gel using natural substrates - maltose and glycogen made it possible to suggest that the enzyme components, formed upon treatment by 3M urea, possess different substrate specificities; one of them splits maltose, but does not split glycogen, while the other splits glycogen having no effect on maltose.

Acknowledgment.

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