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STUDIES ON THE CELL SURFACE MALTASE OF THE RABBIT RENAL CORTEX

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SUMMARY

Membrane vesicles which are derived mainly from the apical membranes of the cells of the renal proximal tubules have been isolated. The effect of exposure to papain on these is to release into the supernatant a soluble fraction which carries the major portion of the maltase activity. An investigation has been made into the nature of this soluble fraction and into the effect of antiserum directed against membrane immunogens on enzymic activity towards small and large substrates.

1. Analysis of the soluble fraction released from the membrane by the action of papain has shown it to consist mainly of protein (74.2% ± 2.1 (4)) with some sialic acid (2.1% ± 0.5 (3)).

2. Electrophoresis of this fraction in polyacrylamide gel at pH 8.6 has revealed four components, all glycoprotein in nature; one of these carried maltase and glucoamylase activities. At least three of the components, including the maltase, formed precipitin arcs with the antiserum raised to whole membrane vesicles.

3. This antiserum brought about precipitation of both the maltase and glucoamylase activities. However, the precipitated maltase retained most (approx. 88%) of its activity whereas the glucoamylase was almost completely inhibited. A similar effect on the respective enzymic activities was observed after treatment of the membrane-bound enzymes with antiserum.

INTRODUCTION

Maltase activity has been demonstrated in the renal cortex by several investigators[1,2], although its physiological role is still not known. Berger and Sacktor[3] first showed that the specific activity of this enzyme increased as morphologically intact proximal tubular brush borders were purified from rabbit tissue. They suggested, therefore, that maltase could be used as a marker enzyme for brush border membrane.

In an attempt to prepare large quantities of proximal tubular plasma membrane for more detailed investigation, a vesicularized membrane fraction was isolated by virtue of its maltase activity[4]. Antisera raised against this fraction were shown

Abbreviation: TEMED, *N,N,N′,N′*-tetramethylethylenediamine.

to react with proximal tubular cell apices, including the brush border region, by the indirect immunofluorescence technique[5]. This demonstrated that the membrane immunogens originated in the brush border, and as the antiserum was capable of specific precipitation of soluble maltase activity, that maltase was also in this region.

The present investigation has been concerned with further characterization of the membrane vesicle fraction, and also of the macromolecular material released from it by papain. It appears that papain cleaves at least four macromolecules from the membrane, all of which are glycoproteins and one of which is maltase. The separated maltase also shows glucoamylase activity. The effect of antiserum to the membrane fraction on maltase and glucoamylase activities is to inhibit the latter almost completely whether the enzyme is membrane bound or solubilized, but to have little effect on maltase activity.

MATERIALS AND METHODS

The isolation of the membrane vesicle fraction from the renal cortex of the rabbit has been described in detail[4]. Its use in raising antisera in guinea pigs has also been described[5].

Preparation of soluble maltase from the membrane vesicles

This was carried out as before using a 5-min incubation with crystalline papain (0.7 mg/ml)[4]. In order to simplify subsequent procedures and to reduce the incidence of fungal infection all column fractionations were in 12 mM potassium phosphate buffer (pH 6.5) containing 0.01% (w/v) thiomersal (B.D.H.), rather than the Krebs–Ringer–bicarbonate buffer used previously[4]. This change did not affect separation and recovery of the macromolecular fraction. It should be noted that it is not possible to concentrate solutions containing thiomersal in the Amicon ultrafiltration apparatus using a PM-10 membrane as there is some retention in the concentrate; the thiomersal must be removed by dialysis and added to the concentrate in an appropriate amount. The residue from papain digestion was washed twice and resuspended in the phosphate–thiomersal buffer.

Assays

Maltase and glucoamylase activities were assayed by the method of Dahlqvist[6]; the substrate for glucoamylase was potato amylose (Sigma) 20 mg/ml in the usual maleate buffer (pH 6.0), but containing 2 mM EDTA.

Protein was determined by the method of Lowry *et al.*[7] using crystalline bovine serum albumin (B.D.H.) as a standard. Sialic acid was assayed by the procedure of Warren[8] using crystalline *N*-acetylneuraminic acid (Sigma) as standard. Hydrolysis at 80 °C for 1 h was used to release *N*-acetylneuraminic acid; any insoluble material was removed by centrifugation before assay. Thiomersal interfered with the Warren procedure and was first removed by dialysis. Dry weights were determined on samples after drying for 15 h at 102 °C. If buffer was present, samples of buffer only were also dried down, and then subtracted.

Polyacrylamide gel electrophoresis

A vertical disc gel electrophoresis apparatus (Shandon) was used as described by Smith[9]. A 5% gel in a glycine buffer was polymerized by addition of ammonium

persulphate and TEMED (*N,N,N',N'*-tetramethylethylenediamine, B.D.H.) in siliconized glass tubes 10 cm in length and with an internal diameter of 6 mm. The procedure was as follows: Stock gel solution: 22.2 g acrylamide monomer (B.D.H.)+0.3 g bisacrylamide (B.D.H.) in 100 ml water. Gel buffer: 0.1 M glycine buffer (pH 8.7) containing 0.01% thiomersal.

For a 5% gel, 6–8 ml gel solution was mixed with 21.8 ml gel buffer and 1.5 ml ammonium persulphate (7.5 mg/ml in water). After deaeration, 0.01 ml TEMED was added with stirring and the gel was immediately transferred to the vertical tubes. Water (20 μl) was layered onto the surface of the gels and after 30 min could be shaken out. The tubes were then inserted into the apparatus and glycine buffer (0.05 M (pH 8.7) containing 0.01% thiomersal) was placed in the upper and lower compartments. Electrophoresis was carried out at 2 mA per gel for 30 min before application of samples in order to remove catalysts. The current was switched off and samples (100 μl containing approx. 25 μg protein, 1 drop glycerol and 1 drop of Bromophenol Blue (1% in buffer)) were placed on top of the gels. Electrophoresis then proceeded at 2 mA per gel for 1.25 h. Gels were removed from the tubes by irrigation with water from a syringe and examined in one of several ways:

(1) Stained for protein with Coomassie Brilliant Blue (Gurr)[10]. Destaining was by immersion in acetic acid–methanol–water (75:50:875, by vol.) for several hours.

(2) Stained for carbohydrate by the periodate–Schiff reagent[11].

(3) Assayed for maltase and/or glucoamylase. Gels were measured and sliced with a razor blade into 0.3-cm lengths. Each slice was placed in 0.05 ml phosphate buffer (0.2 M (pH 6.2) containing 0.01% thiomersal). The maltase assay was then carried out after addition of 0.1 ml substrate. In order to detect glucoamylase it was necessary to allow the gel slices to stand in the phosphate buffer overnight. This presumably reflects the inability of the high molecular weight substrate to diffuse into the gel.

(4) Immunological activity: gels were sliced as for enzyme assay and the slices were inserted vertically into troughs cut into agar. A drop of the phosphate buffer (0.2 M (pH 6.2)) was then allowed to run over each slice into the trough and diffusion took place for about 5 h. Antiserum was then placed in a central trough cut parallel to the gel troughs and the plate was left to develop in a humidity cabinet. Plates were stained, when required, by Amido Black[12].

Effect of antiserum on maltase and glucoamylase activities

The preparation of the antiserum and the construction of a precipitin curve for maltase was essentially as described previously[5]. In that technique the supernatant and the precipitated maltase activities were assayed in the presence of serum. A major difficulty arose in using this method for glucoamylase, as the presence of serum caused a large stimulation of enzymic activity. This has been reported also for α-amylase[13]. Therefore, in order to assess the effects of antibody on glucoamylase activity, the antigen–antibody precipitates were collected, washed twice with cold 12 mM potassium phosphate buffer (pH 6.5) containing 0.01% thiomersal, resuspended in 0.1 ml of this and assayed. Supernatants were also assayed, bearing in mind the stimulatory effect of their serum content.

A similar technique was used to measure the effect of antiserum on membrane-

bound enzymes; it was necessary to incubate as for the soluble enzymes and then to centrifuge the membrane–antibody complex in the Sorvall SS-1 centrifuge at 12520 × *g* and 4 °C for 50 min. The pellets were washed twice with the cold phosphate–thiomersal buffer before resuspension for assay. Supernatants were assayed to check for any loss of enzymes.

RESULTS

Papain digestion

A summary of the effect of papain on several different preparations of the membrane vesicles is given in Table I. It is clear that a fraction rich in protein and sialic acid has been split off the vesicles by proteolysis, leaving a residue which still contains considerable amounts of protein and sialic acid although reduced as percentages of dry weight. The total recovery of protein after collection of the macromolecular fraction from the column, together with the residue, was always in the range 48–55%. As the recovery of the membrane-bound enzyme, trehalase, was always >85%, the majority of the losses must have occurred in the soluble fraction. Comparison of $A_{280\ nm}$ values of enzyme control and test elutions on the Sephadex G-100 column suggests that some material was being eluted in the papain and/or buffer peaks. There was a similar loss of sialic acid and this suggests that low molecular weight glycoproteins have been produced by papain.

TABLE I

EFFECT OF PAPAIN ON THE MEMBRANE VESICLES

Percentages quoted are of dry weights; ranges are standard errors of the mean, with the number of readings in parentheses

	% protein	*% sialic acid*
Membrane vesicles	53.8 ± 2.4 (4)	0.69 ± 0.09 (4)
Papain digest		
Supernatant	72.9 ± 1.4 (3)	2.1 ± 0.5 (3)
Residue	43.4 ± 1.7 (4)	0.51 ± 0.04 (4)

Heterogeneity of the soluble fraction

The macromolecular components released from the membrane vesicles by papain were examined by disc electrophoresis in 5% polyacrylamide gel. The results of staining such a gel for protein are shown in Fig. 1. A similar pattern was obtained for all the soluble fractions examined. The periodate–Schiff reagent reacted with all four bands demonstrating that they also contained carbohydrate.

After cutting the gel into slices and assaying for maltase and glucoamylase activities, it was found that both enzymes were located exclusively in Band 1. Also, the relative activities were the same as in the original soluble fractions, suggesting that the two enzymic activities are carried by the same protein.

After insertion of the gel slices into agar and allowing the contents to diffuse against an antiserum raised to the membrane vesicles, several arcs of precipitation

Fig. 1. Polyacrylamide gel electrophoresis of the material solubilized from the membrane vesicles by papain. Electrophoresis was in 5% gel at pH 8.6 for 1.25 h with the anode on the right. Coomassie Blue was used to detect protein.

were seen (Fig. 2). The most reactive was formed by Band 4 but arcs were clearly visible from Bands 1, 2 and 3. This was deduced from measurement of the gel before and after staining, when it was found that Band 1 ≡ Slice 5, Band 2 ≡ Slices 9 and 10, Band 3 ≡ Slices 11 and 12 and Band 4 ≡ Slices 16, 17 and 18. Thus it appears that all four components of the soluble fraction were involved in the immunogenicity of the original membrane vesicles.

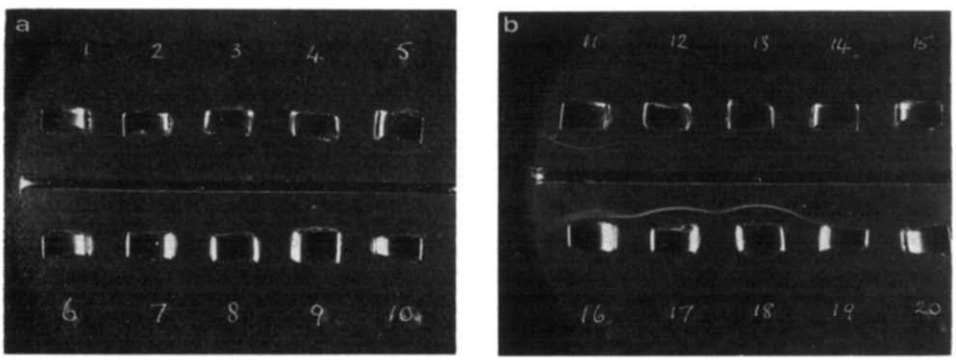

Fig. 2. Immunodiffusion of the contents of slices from the polyacrylamide gel run of material solubilized from membrane vesicles by papain. The gel was sliced into 0.3-cm widths and inserted into slots cut in the agar. Slices were moistened with buffer periodically and after 5 h antiserum raised to membrane vesicles was placed in the central trough. Immunodiffusion was allowed to proceed at room temp. in a humidity cabinet for 4 days. The slices are numbered from the origin.

Effect of antiserum on enzymic activities

The precipitation of maltase activity by the antiserum raised to the membrane vesicles has been reported[5]. In order to demonstrate specific precipitation of glucoamylase activity by the antiserum, aliquots (20 μg protein) of the soluble fraction were incubated as described in Materials and Methods with aliquots (0.08 ml) of antiserum, normal guinea pig serum and buffer only. After centrifugation, half of the supernatant and the pellet resuspended in the remaining supernatant were assayed for glucoamylase. The results are shown in Table II. It is clear that the antiserum has brought about complete precipitation of glucoamylase activity, whereas normal serum has had no effect, distribution of enzyme between supernatant and supernatant *plus* pellet being equal. However, it can also be seen that the presence of serum has caused a large stimulation of total enzyme activity, thereby obscuring any inhibition by antibody molecules.

A further experiment was carried out in which the immune precipitates were centrifuged and washed before assay, as described in Materials and Methods. The results are shown in Table III, together with an experiment run in parallel for maltase.

TABLE II

EFFECT OF ANTISERUM RAISED TO MEMBRANE VESICLES ON THE DISTRIBUTION OF GLUCOAMYLASE ACTIVITY BETWEEN EQUAL VOLUMES OF SUPERNATANT AND SUPERNATANT *PLUS* RESUSPENDED PELLET

Enzymic activities are reported as μg glucose produced/h.

Enzyme treated with	*Glucoamylase activity*		
	Supernatant	*Supernatant + pellet*	*Total*
Antiserum	0	153.2	153.2
Normal guinea pig serum	83.6	91.4	175.0
Buffer			54.0

TABLE III

EFFECT OF ANTISERUM RAISED TO MEMBRANE VESICLES ON ENZYMIC ACTIVITIES OF SOLUBLE MALTASE AND GLUCOAMYLASE

Enzymic activities are reported as μg glucose produced/h.

Volume of antiserum (ml)	*Glucoamylase*		*Maltase*	
	Supernatant	*Pellet*	*Supernatant*	*Pellet*
0.02	31.6	0	204	13.0
0.05	0	5.0	0	176
0.08	0	3.3	0	157
0.08 ngps*	95.2	0	223	0
0	28.2		178	

* ngps, normal guinea pig serum.

TABLE IV

EFFECT OF ANTISERUM RAISED TO MEMBRANE VESICLES ON ENZYMIC ACTIVITIES OF MEMBRANE-BOUND MALTASE AND GLUCOAMYLASE

Enzymic activities are reported as μg glucose produced/h.

Volume of antiserum (ml)	*Glucoamylase*	*Maltase*
0.075	4.8	234
0.15	3.8	231
0.15 ngps*	25.2	260
0	19.4	274

* ngps, normal guinea pig serum.

It can be seen that the activity of glucoamylase in the immune precipitate has been decreased considerably. Activity after incubation with 0.08 ml antiserum has been reduced to 12% of that of the stored enzyme. Maltase, however, shows 88% of the activity of the stored enzyme after incubation with 0.08 ml antiserum.

The effect of antiserum on membrane-bound enzyme activities was examined as described in Materials and Methods and the results are shown in Table IV. The results demonstrate a similar effect to that on the soluble enzyme: maltase retains 90% of the activity of that of enzyme incubated with normal serum, whereas glucoamylase retains 15%.

DISCUSSION

The membrane vesicles have been shown to be derived from the apical regions of the cells of the proximal tubules[5]. Since the preparative procedure has included both exposure to hypotonic solutions and extensive washings in various buffers, any loosely bound protein should have been removed. Analysis for protein and sialic acid (53.8% and 0.69% of dry weight, respectively) demonstrates a close similarity between these membranes and a preparation of rat liver plasma membrane (58% protein and 0.56% sialic acid) analyzed by Emmelot and Bos[14]. These authors also used hypotonicity and extraction with salt solutions to remove loosely bound protein.

The effect of papain on the vesicles is to bring about the solubilization of a glycoprotein fraction, leaving a membrane residue depleted of protein and sialic acid. The glycoprotein fraction was resolved on polyacrylamide gel electrophoresis into 4 components, the slowest band being maltase. Papain has been used frequently to solubilize disaccharidases and other enzymes from the intestinal brush border[15–18]. Alpers[19] studied the effect of papain on rat intestinal mucosa and electrophoresed the soluble products on polyacrylamide gel; maltase activity was demonstrated in a slow well-demarcated band. Forstner[20] obtained similar results and demonstrated three main bands which stained with periodate–Schiff; he also noted that there was no release of free protein into the supernatant. Obviously there is considerable structural analogy between the renal and intestinal brush borders.

The activity of the renal maltase corresponds to that of intestinal maltase 1 and 2 in Semenza's[21] classification as it has no sucrase activity[3,5] and little or no isomaltase activity[3]. Isomaltase activity was checked on the membrane vesicles and found to be 0.015 times maltase specific activity. The properties of the rat intestinal heat-stable maltase with associated glucoamylase activity have been described by Kolínská and Kraml[22]. In this enzyme the glucoamylase activity represents approx. 0.7 times the maltase activity whereas the figure for the renal glucoamylase described here is 0.2 times the maltase activity.

Immunodiffusion of the four glycoprotein components of the soluble fraction against the antiserum raised to the membrane vesicles demonstrated that all four formed precipitin arcs. The immunogenicity of the components suggests that they were oriented originally on the membrane in exposed positions.

The effect of antiserum on the different enzymic activities of the renal maltase follows a previously described pattern. The ability of antibodies to block access of large substrate molecules to the active site while allowing free access to small substrates has been demonstrated notably for neuraminidase and ribonuclease[23].

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