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GLYCOSIDASES OF THE GUINEA PIG BRUSH BORDER MEMBRANE

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Summary

The separation by polyacrylamide gel electrophoresis and subsequent enzymatic analysis of the components of the guinea pig intestinal brush border membrane revealed the presence of three enzyme complexes: maltase-glucoamylase, maltase-sucrase-glucoamylase and maltase-sucrase. Additional bands possessing lactase, trehalase and alkaline phosphatase activity were identified but no phlorizin hydrolase or palatinase was detectable. After exposure to strong dissociating conditions the bands possessing enzymatic activity were either absent or greatly reduced in intensity.

Introduction

The development of a gel electrophoresis system capable of separating intestinal brush border membrane components, which retained their enzymatic activity, made it possible to ascribe various intestinal enzymatic activities to specific protein or glycoprotein bands present on a gel [1].

Earlier work on lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23) had shown it to be strongly associated with phlorizin hydrolase (phloretin glucosidase, EC 3.2.1.62), and similarly sucrase (sucrose α-glucohydrolase, EC 3.2.1.48) was tightly bound to isomaltase (oligo-1,6-glucosidase, EC 3.2.1.10) [2,3]. On gels of human brush border membrane two peaks of maltase activity (α-glucosidase, EC 3.2.1.20) were identified, one was associated with glucoamylase (exo-1,4-α-glucosidase, EC 3.2.1.3) and the other with sucrase and isomaltase. Lactase and phlorizin hydrolase were found as a single band between the two maltases, and trehalase (α,α-trehalase, EC 3.2.1.28) was present as a single fast band. With improvements in the technique and its application to biopsies from patients with disaccharide intolerance syndromes, it was shown that the reduction or absence of an individual band resulted in the diminution or lack of the corresponding enzymatic activity and hence intolerance to a

specific sugar [4,5]. These results effectively demonstrated that, at least for lactase-phlorizin hydrolase and for sucrase-isomaltase, a single stained band on a gel represented a single enzymatic complex and not a group of unrelated proteins migrating together. Using similar methods we now report the finding of a more complex pattern of enzymatic activity in guinea pig brush border membranes.

Methods

Guinea pigs (3—26 weeks old, weight 150—850 g) were fasted for 24 h, anaesthetized, and the first third of proximal small intestine removed. This was opened, rinsed with ice-cold 0.15 M saline and the mucosa scraped off. Purified brush border membranes, isolated by the Schmitz method [6], were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate according to Laemmli [7]. For gels on which enzymatic activities were measured, the membrane sample was mixed with the Laemmli sample buffer but not boiled. After 15 min at room temperature with occasional mixing, the solubilized sample was centrifuged at 10 000 × *g* for 2 min and the supernatant layered onto the gel. For complete dissociation of the membrane proteins, with the concomitant loss of enzymatic activity, the supernatant was boiled for 5 min. After electrophoresis the gels were sliced longitudinally, one half was stained, the other half was sliced into 0.5-mm sections which were eluted with distilled water for 18 h at 4°C. Enzymatic activities in the eluates were measured with the relevant substrates as described previously [4,8—11]. Protein was assayed by the Lowry et al. procedure [12].

Results and Discussion

Although the Schmitz technique was developed for human material, it was equally effective in the preparation of guinea pig brush borders. The purification factor for sucrase was greater than 6-fold with a total recovery in excess of 80%. After gel electrophoresis the stained gel and the enzyme distribution pattern differed significantly from those published for human or hamster brush border [1,13]. Fig. 1a shows the protein scan of a stained gel. Fig. 1b shows the corresponding enzyme pattern. Stained bands I, II and IV coincided with three regions of maltase activity, the latter two also possessed sucrase activity. In addition glucoamylase was present in the two slower regions of maltase activity; stained bands I and II. On a semi-quantitative basis the ratios of the co-migrating enzymes varied in the three complexes. Using palatinose as a substrate for isomaltase [14], we were unable to demonstrate palatinose hydrolase activity, so the presence in guinea pig of a sucrase-isomaltase complex similar to the human one remains to be established. Lactase activity, detected only in young animals, appeared as a single peak (stained band III) devoid of phlorizin hydrolase activity. The high mobility of the lactase band, relative to the human lactase-phlorizin hydrolase enzyme, may be tentatively attributed to the absence of a phlorizin hydrolase subunit from guinea pig lactase. Trehalase (stained band VIII) and alkaline phosphatase (stained band V) were both found on the gel as single bands devoid of additional enzymatic activities.

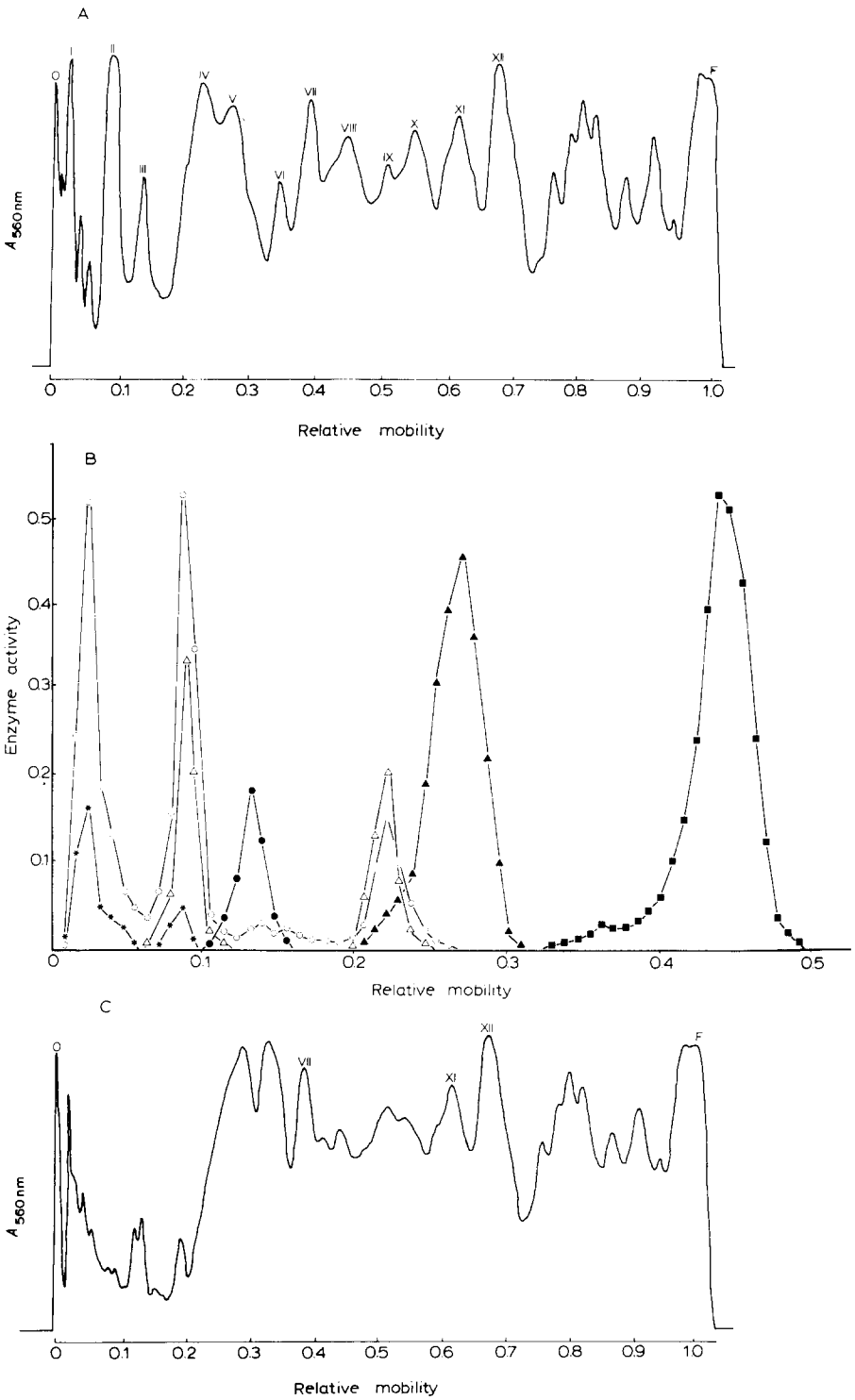

Fig. 1. Polyacrylamide gel electrophoretic pattern on Laemmli gels of purified brush border membranes from a 4-week-old guinea pig 60 μg protein solubilized in sample buffer for 15 min at room temperature. (A) Protein stain scan. (B) Corresponding distribution pattern of enzyme activity in arbitrary units. (C) Protein stain scan of sample boiled for 5 min in sample buffer, O, Origin, F. front. ○———○, maltase; △———△, sucrase; *———*, glucoamylase; ●———●, lactase; ■———■, trehalase; ▲———▲, alkaline phosphatase.

These findings raise several fundamental questions concerning the structure of the glycosidase enzymes and the effectiveness of gel electrophoresis in separating them. The presence of a stained band coincident with multiple enzymatic activity may be interpreted in several ways. It could be a single enzyme with a catalytic site of broad specificity, or an enzyme complex with several catalytic sites, or several separate enzymes which only fortuitously co-migrate. Furthermore, artifacts arising from the sample preparation or during electrophoresis cannot be excluded, but the fact that sucrase-isomaltase and lactase-phlorizin hydrolase complexes have been isolated from other species by different methods provides evidence against this. Although intestinal glycosidases do exhibit some flexibility in their substrate requirements [15,16], there is no evidence for a single enzyme capable of simultaneously hydrolysing sucrose, maltose and glucoamylose. In other species sucrase-isomaltase [14,17] and maltase-glucoamylase [16] activities have been shown to be due to two separate catalytic sites present as a single complex. To investigate this possibility further, gels were run of samples which had been boiled in 2% sodium dodecyl sulphate and 5% mercaptoethanol. This treatment predictably destroyed all enzymatic activity and altered the position and intensity of most of the higher molecular weight bands (Fig. 1c). None of the peaks to which enzymatic activity had been ascribed remained unaltered. Only bands VII, XI, XII and the lower molecular weight peaks beyond XII were unchanged. New peaks appeared between bands V and VII with a minor one between bands IX and X. Although the interpretation of these results remains equivocal, several general conclusions may be drawn.

The presence of residual bands at positions I and III demonstrates that a single sharp band may comprise several superimposed bands. Nevertheless, the almost total disappearance of band II shows that this is not always so. As there was no increase in intensity of a band previously associated with glycosidase activity it is unlikely that any of the higher molecular weight enzymes are polymers of smaller ones. Additional evidence for this is provided by the different ratios of activities for the individual components in each complex.

However, since several of the glycosidase peaks showed multiple activities, they could comprise a mixture of similar basic subunits in different ratios. If this were so, it is surprising that we did not observe on gels of unboiled samples three low molecular weight bands each possessing a single hydrolytic activity against sucrose, maltose or glucoamylose. If they had occurred, such bands would be expected to show an increased intensity on gels of boiled, reduced samples. The possibility remains that some of the stained bands do represent enzyme subunits inactivated by separation from the complexes. Thus it would appear that the multiple glycosidases I, II and IV are separate structural and functional entities, probably representative of the in vivo situation, rather than random aggregates. The presence of residual bands at positions previously associated with enzymatic activities makes further interpretation difficult. Moreover, it is possible that a small amount of active enzyme, detectable by its hydrolysis of substrate, may not be visualized as a stained band. The situation would be improved if a purer brush border preparation giving fewer bands were to be used.

The recent improvements in the separation of brush border from basolateral

membranes and core proteins [18,19] should give a gel with fewer bands and thus easier to interpret. For the further characterisation of the proposed enzyme complexes conventional large scale purification procedures will be necessary to yield sufficient enzyme for detailed analyses.

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References

1 Maestracci, D., Preiser, H., Hedges, T., Schmitz, J. and Crane, R.K. (1975) Biochim. Biophys. Acta 382, 147—156
2 Colombo, V., Lorenz-Meyer, H. and Semenza, G. (1973) Biochim. Biophys. Acta 327, 412—424
3 Braun, H., Cogoli, A. and Semenza, G. (1975) Eur. J. Biochem. 52, 475—480
4 Freiburghaus, A.U., Schmitz, J., Schindler, M., Rotthauwe, H.W., Kuitunen, P., Launiala, K. and Hadorn, B. (1976) N. Engl. J. Med. 294, 1030—1032
5 Preiser, H., Menard, D., Crane, R.K. and Cerda, J.J. (1974) Biochim. Biophys. Acta 363, 279—282
6 Schmitz, J., Preiser, H., Maestracci, D., Gosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98—112
7 Laemmli, U.K. (1970) Nature 227, 680—685
8 Messer, M. and Dahlqvist, A. (1966) Anal. Biochem. 14, 376—392
9 Leese, H.J. and Semenza, G. (1973) J. Biol. Chem. 248, 8170—8173
10 Asp, N.G. and Dahlqvist, A. (1972) Anal. Biochem. 47, 527—538
11 Garen, A. and Levinthal, C. (1960) Biochim. Biophys. Acta 38, 470—483
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
13 Critchley, D.R., Howell, K.E. and Eicholz, A. (1975) Biochim. Biophys. Acta 394, 361—376
14 Cogoli, A., Eberle, A., Sigrist, H., Joss, C., Robinson, E., Mosimann, H. and Semenza, G. (1973) Eur. J. Biochem. 33, 40—48
15 Kraml, J., Kolinska, J., Ellederova, D. and Hirsova, D. (1972) Biochim. Biophys. Acta 258, 520—530
16 Sivakami, S. and Radhakrishnan, A.N. (1976) Biochem. J. 153, 321—327
17 Yamashiro, K.M. and Gray, G.M. (1970) Gastroenterology 58, 1056
18 Michell, R.H., Coleman, R. and Lewis, B.A. (1976) Biochem. Soc. Trans. 4, 1017—1020
19 Lewis, B.A., Elkin, A., Michell, R.H. and Coleman, R. (1975) Biochem. J. 152, 71—84