SEPARATION OF HUMAN SMALL-INTESTINAL SUCRASE FROM ISOMALTASE

N.-G. ASP and A. DAHLQVIST
Department of Nutrition, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund 7, Sweden

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1. Introduction

Both heat inactivation experiments and mixed substrate incubations have demonstrated that the human intestinal sucrase and isomaltase activities are exerted by different enzymes [1–3]. Separation techniques, such as gel filtration [4], ion-exchange chromatography [5], and gradient ultracentrifugation [6] have generally failed to separate the two enzymes, but shown that they occur bound together in a complex in solubilized mucosal homogenates. Recently, however, Yamashiro and Gray [7] mentioned in an abstract that the complex could be split by dialysis against 6 M urea with 0.01 M mercaptoethanol. Free isomaltase and sucrase could then be separated by gradient ultracentrifugation. To date, however, no further experimental details have been published.

In Sephadex G-200 chromatograms an additional sucrase peak (sucrase-1) without concomitant isomaltase activity has been reported [4]. Sucrase-1 was originally interpreted as a separate enzyme different from sucrase in the sucrase–isomaltase complex (sucrase-2) [4, 8], but is now believed to arise from the complex by inactivation or removal of the isomaltase moiety [5, 9, 10].

The rabbit small-intestinal sucrase–isomaltase complex has been isolated and characterized extensively in another laboratory [9–12]. The purification procedure is based primarily on the enzyme–substrate-like interaction with Sephadex, which results in an adsorption and retardation of the solubilized complex on Sephadex G-200 columns. The same purification procedure has also been utilized for an immunochemical study of the human intestinal sucrase–isomaltase complex [13]. Recently the separation of free rabbit isomaltase after inactivation and separation of the sucrase moiety by incubation at pH 9.2 was reported [12, 14].

The present communication reports the appearance of free sucrase and isomaltase, both in active form, in addition to the sucrase–isomaltase complex, after papain solubilization of human small-intestinal mucosal homogenates according to the procedure usually used [15]. Separation of the different components is carried out with gel filtration on Bio-Gel P-300, followed by affinity chromatography on Sephadex G-200.

2. Methods and results

A mucosal homogenate from the proximal jejunum, obtained surgically and prepared as described previously [16], was ultracentrifuged 100 000 *g* for 60 min. The supernatant contained about 10% of the disaccharidase activities. Chromatography of the supernatant on Bio-Gel P-300 at room temperature gave one small peak of sucrase and isomaltase activity at the void volume and a major peak with $K_{av} = 0.08$ (not illustrated). The sediment was then treated with papain in potassium phosphate buffer [15], which made about 90% of the originally particle-bound maltase, isomaltase and sucrase activities soluble after another ultracentrifugation.

Chromatography of the papain solubilized material on a Bio-Gel P-300 column is shown in fig. 1a. A major peak containing about 80% of the sucrase and isomaltase activities was eluted with $K_{av} = 0.08$. This was obviously the sucrase–isomaltase complex. The remaining 20% of the different α-glucosidase activities were eluted in a second peak with $K_{av} = 0.18$, indicating a considerably lower molecular weight. Thus we suspected that the second peak contained free sucrase and free isomaltase and tried to separate the two ac-

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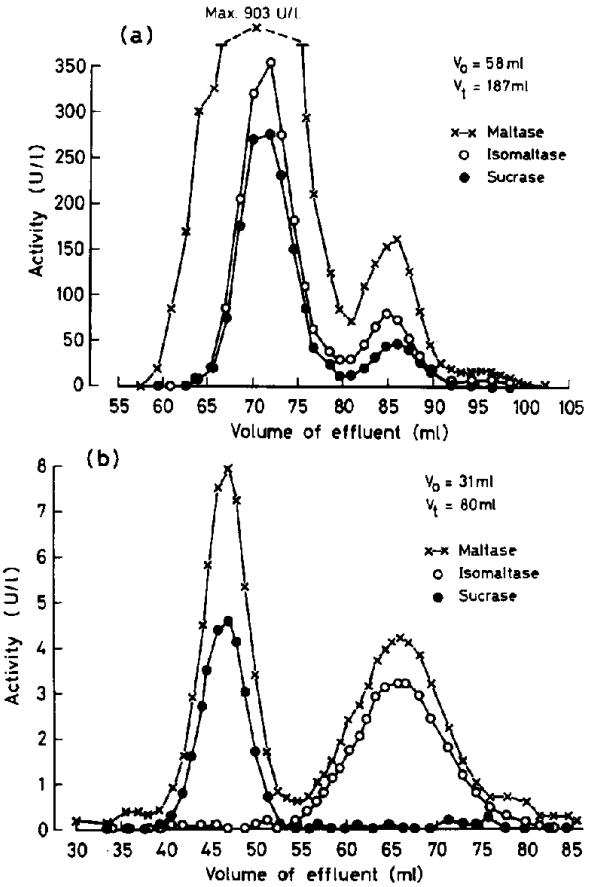

Fig. 1. a) Separation of papain-solubilized material from human jejunal mucosa on a Bio-Gel P-300 column (length 95 cm, diameter 1.5 cm; buffer 0.01 M Na-malate pH 6 with 0.077 M NaCl and 0.077 M KCl). Two peaks with sucrase, isomaltase and maltase activity were separated; b) Re-chromatography of one fraction (86.6–87.8 ml) of the second peak in fig. 1a on a Sephadex G-200 column (length 95 cm, diameter 1 cm; buffer as in fig. 1a). The sucrase was completely separated from the isomaltase and both peaks had maltase activity.

tivities on Sephadex G-200, which is known to retard intestinal isomaltase activity strongly [9]. The result is shown in fig. 1b. Complete separation of the sucrase from the isomaltase was obtained. Both peaks had maltase activity. The isomaltase peak had also glucoamylase activity. The K_{av} of the sucrase was 0.26 which is in agreement with the K_d-value of 0.23 reported for sucrase-1 [7]. Free glucose appeared in the eluate between 60 and 85 ml with maximum at 73 ml.

3. Discussion

These results show that the solubilization with papain released not only the sucrase–isomaltase complex as previously known, but also some free sucrase and free isomaltase from the membrane fragments of human small-intestinal homogenate. Thus, the enzyme peak which in literature has been named sucrase-1 is most probably free sucrase. The free isomaltase, however, cannot be separated from the sucrase–isomaltase complex by Sephadex G-200 chromatography, since both are interacting with the gel in a similar way and are therefore retarded. This is why chromatography on Bio-Gel P-300 must be included as a first separation step, in the way we have described above.

The separation of sucrase from isomaltase in a preparative scale allows an individual characterization of the two enzymes, which is in progress.

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