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DELETION OF ENZYME PROTEIN FROM THE BRUSH BORDER MEMBRANE IN SUCRASE—ISOMALTASE DEFICIENCY

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Summary

Acrylamide gel electrophoresis of brush border membranes from two siblings with sucrase—isomaltase deficiency revealed the absence of the protein band which is normally associated with the sucrase—isomaltase complex. Brush border enzyme activities other than sucrase and isomaltase were unimpaired and reflected a gel pattern comparable to that obtained with membranes from control subjects.

Sucrose—isomaltose intolerance [1] is an hereditary disorder [2] ordinarily rare in occurrence [3—5] and characterized by a total deficiency of brush border membrane sucrase activity and a reduction of brush border membrane isomaltase activity to trace amounts in most patients. In a recent reports this brush border membrane enzymatic deficiency was studied by immunofluorescent techniques [6]. It was concluded that "the enzyme protein was indeed present" but in a structurally aberrant form with impaired catalytic activity. We have recently developed methods for the isolation of human intestinal brush border membranes and the characterization of the protein components of these membranes [7,8]. Technical extension of these methods has now permitted a comparative study of biopsies from two normal subjects and two patients with sucrase—isomaltase deficiency. Our findings indicate that these enzyme proteins are not present in the brush border membrane in the diseased state.

Two normal adult subjects and two patients with sucrase—isomaltase deficiency, ages 11 and 13 years, were studied. Both patients had a flat

sucrose-tolerance test but a normal response to oral glucose. Peroral jejunal biopsies showed normal histology in all cases. Portions of the biopsies were frozen, airmailed to New Jersey and used for the isolation of purified microvillus membranes as previously described [7] as well as for enzyme assay. Disaccharidases, namely sucrase (sucrose α-glucohydrolase, EC 3.2.1.48), isomaltase (oligo-1,6-glucosidase, EC 3.2.1.10), maltase (α-D-glucoside glucohydrolase EC 3.2.1.20), lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23) and trehalase (EC 3.2.1.28) were assayed according to Dahlquist [9], leucylnaphthylamide hydrolysing activity according to Goldbarg and Rutenburg [10], alkaline phosphatase (EC 3.1.3.1) according to Eichholz [11], glucoamylase (exo-1,4-α-glucosidase, EC 3.2.1.3), using soluble starch as substrate, according to Schlegel-Haueter et al. [12]. γ-Glutamyltransferase (EC 2.3.2.2) according to Naftalin et al. [13] and protein according to Lowry et al. [14]. Acrylamide gel electrophoresis was carried out as follows: Multiple-sample gel slabs were cast in a 4.5, 8% acrylamide monomer gradient in Tris—sulfate buffer, pH 9.0, containing 0.1% sodium dodecylsulfate. After formation of wells with 8% gel, the solubilized membranes (2% sodium dodecylsulfate) were suspended in buffered sucrose, layered into the wells and capped with 8% gel. Electrophoresis was performed using 0.065 M Tris—borate, pH 9.0, as upper and lower buffer and a pulsed constant-power supply (280 V and 1.0 μF). The pulses per minute were raised from 75 to 300 over a period of 30 min and the electrophoresis was allowed to continue for a total run of 70 min. The upper buffer contained a trace of bromophenol blue and 0.1% sodium dodecylsulfate. The slabs were fixed in 12.5% trichloroacetic acid, stained for 2 h at 65 °C in 0.25% Coomassie Brilliant Blue and destained in 10% acetic acid at 65 °C.

Data from the various enzymatic assays of the subjects studied are presented in Table I. The values of the normals agree with our previous experience. The patients, J.B. and M.B., who are siblings, however, have no sucrase and only trace isomaltase activity and thus represent typical cases of congenital sucrase—isomaltase deficiency [2,3,6,15]. Maltase activity in the patients is low as expected. Upon heat inactivation at 54 °C for 60 min,

TABLE I

ENZYME SPECIFIC ACTIVITIES IN HOMOGENATES OF PERORAL JEJUNAL BIOPSIES

Specific activity in I.U./g protein

Enzymes	Patients		Controls	
	J.B.	M.B.	O.P.	W.Y.
Sucrase	0	0	36.1	31.7
Isomaltase	0.4	0	20.7	17.5
Maltase	30.7	25.5	119.5	133.4
Glucoamylase	8.9	7.5	7.8	8.5
Lactase	48.7	42.5	22.4	16.8
α,α-Trehalase	24.8	30.4	12.6	14.6
Alkaline phosphatase	170.8	201.4	161.0	69.0
Leucylnaphthylamide hydrolysing activity	43.0	34.3	56.7	38.3
γ-Glutamyltransferase	22.7	18.9	25.3	26.9

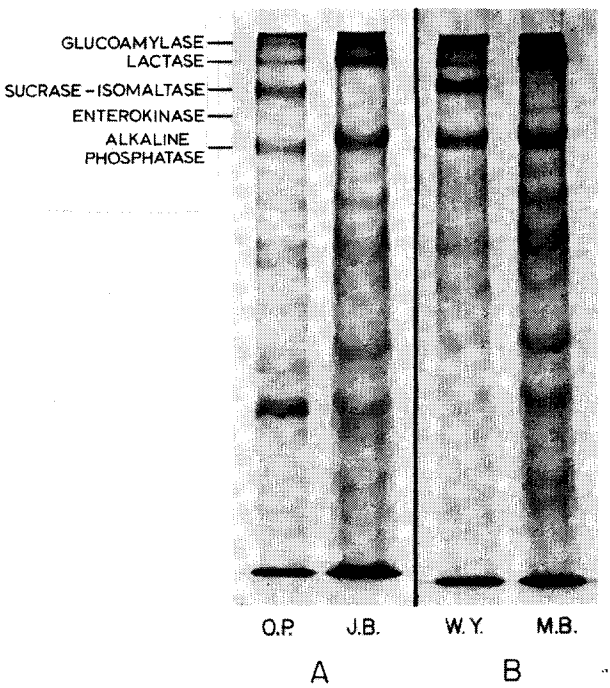

Fig.1. Acrylamide gel electrophoresis patterns obtained as described in the text. In A, membranes from normal subject O.P. and patient J.B. were run concurrently. In B, membranes from normal subject W.Y. and patient M.B. were run concurrently.

87%—91% of the maltase activity was retained as compared to 26% in the normal. These results are in agreement with those by Auricchio et al. [15] and confirm earlier findings [3] which attribute the maltase activity in patients with sucrase—isomaltase deficiency to heat-resistant maltase; that is, Maltase II and III of Dahlquist [16] or 1 and 2 of Semenza et al. [17]. Activity of glucoamylase in the patients was found to be normal and not decreased as reported by Eggermont and Hers [18]. Our results with glucoamylase are supported by Kelly and Alpers [19] who demonstrated the similarity of purified glucoamylase (stable at 54 °C) to heat-resistant Maltase II from human intestine. Also, Kelly and Alpers found hydrolysis of isomaltose by glucoamylase which may explain the trace of isomaltase activity found in these patients. The activities of lactase and α,α-trehalase were elevated. Alkaline phosphatase and peptidase activities were within the normal range.

The protein patterns obtained by acrylamide gel electrophoresis are shown in Fig. 1. From the top, the bands in the normal correspond to maltase—glucoamylase, lactase—phlorizin hydrolase, sucrase—isomaltase, enterokinase and alkaline phosphatase, respectively, based upon assays carried out in this laboratory by Maestracci on water extracts of serial slices (1 mm thick) of gels which were electrophoretically developed but not

stained with Coomassie Brilliant Blue. A preliminary report has been made [20]. The assay methods used were as referenced above except for enterokinase (enteropeptidase, EC3.4.4.8) which was as given by Schmitz et al. [21].

In the protein patterns from the patients the normally very intense band associated with sucrase—isomaltase is absent. The bands related to other enzyme activities are all visible and in some instances more intense in the gel pattern from the patients than in those from the controls. The gels were carefully examined and no new band could be found in the protein pattern from the patients.

The absence of the sucrase—isomaltase proteins could not be anticipated from the findings of Dubs et al. [6]. These authors demonstrated an antigen to sucrase—isomaltase antibody in the brush border region in situ by immunofluorescence and concluded that the enzyme protein was present but inactive. We cannot find the protein in isolated membranes. The reason for the difference is not clear. The other brush border enzymes display unimpaired activity and may be assumed to be correctly incorporated into the membrane. Consequently, defective incorporation of an inactive sucrase—isomaltase protein into the membrane leading, perhaps, to its loss during membrane preparation seems unlikely. The possibility should be considered that a fragment of the sucrase—isomaltase complex, responsible for the stability of the association of these two enzymes and/or their binding into the membrane and not inactive enzyme, per se, is an important contributor to antigenicity such as detected by Dubs et al. [6].

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