Physicochemical characterization of human intestinal lactase

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Human lactase was isolated from solubilized small-intestinal brush-border membranes by a combination of chromatography on concanavalin A–Sephadex, Bio-Gel 1.5m and chromatofocusing, with a yield of approx. 1% and a 750-fold purification. The enzyme appeared to be homogeneous on SDS/polyacrylamide-gel electrophoresis under both reduced and non-reduced conditions, with an apparent M_r of approx. 170000. On gel filtration, however, it displayed an apparent M_r of approx. 380000. The protein had a pI of 4.8, as judged by the chromatofocusing experiment, and had a lactase activity whose optimum is at pH 6.0. In addition to the β -galactosidase activity, the protein also hydrolysed to various extents cellobiose, phlorizin, *p*-nitrophenyl β -D-galactoside, *p*-nitrophenyl β -D-glucoside, *o*-nitrophenyl β -D-galactoside and *o*-nitrophenyl β -D-fucoside. Antisera had been raised against the purified enzyme in two rabbits. One of the antibody populations could inhibit the enzyme in a concentration-dependent manner. This antibody population was used to set up an antibody-bound Sephadex column for the use in an immunoaffinity purification of lactase from crude intestinal homogenate. A partially purified preparation of lactase could thus be obtained. The antibody population was also used to set up a radioimmunoassay for quantifying the enzyme. The competition assay could detect about 0.5 μ g of lactase protein/ml.

INTRODUCTION

Lactose is the principal source of energy in milk. The enzyme responsible for hydrolysing lactose is a β -galactosidase (EC 3.2.1.23) believed to reside in the brush-border membrane of the small-intestine villi. There are three different β -galactosidases in the human small intestine. One of these is lysosomal in origin, and is therefore presumably not involved in digesting lactose on the brush border. The other two enzymes are membrane-bound, and only one of them is able to utilize lactose as the substrate, whereas the other hydrolyses hetero- β -galactosides only (Gray & Santiago, 1969; Asp *et al.*, 1969; Asp, 1971). These earlier studies, however, utilized only crude particulate and supernatant fractions of intestinal homogenate rather than isolated subcellular components as their source of starting materials. More recently, partially purified lactase preparations were used as immunogens to produce either polyclonal antibodies (Skovbjerg *et al.*, 1981) or monoclonal antibodies (Potter *et al.*, 1985). These antibodies were then used to carry out one-step immunoaffinity chromatography for the purification of the enzyme. I report here the preparation of lactase from human small-intestinal brush-border membrane fragments by using conventional column-chromatographic methods, and some of the physicochemical characteristics of the purified enzyme.

MATERIALS AND METHODS

Materials

p-Nitrophenyl β -D-galactoside and *o*-nitrophenyl β -D-galactoside were obtained from Sigma (St. Louis, MO, U.S.A.) or Koch–Light (Haverhill, Suffolk, U.K.). *o*-Nitrophenyl β -D-fucoside, phloretin 2'- β -D-glucoside (phlorizin), cellobiose, bovine serum albumin, phenylmethanesulphonyl fluoride, benzamidinium hydrochloride, glucose oxidase, horseradish peroxidase, Triton X-100, α -methyl D-mannoside, Coomassie Brilliant Blue R,

CNBr, chloramine-T, glycylglycine hydrochloride, jack-bean meal and Blue Dextran 2000 were also obtained from Sigma. *o*-Dianisidine and lactose were from BDH (Poole, Dorset, U.K.). Complete Freund's adjuvant and Bio-Gel 1.5m were from Bio-Rad (Richmond, CA, U.S.A.). Sephadex 4B, concanavalin A-bound Sephadex 4B, PBE 94 and Polybuffer 74 were obtained from Pharmacia (Uppsala, Sweden). Reagents used for SDS/polyacrylamide-gel electrophoresis were products of Eastman Kodak (Rochester, NY, U.S.A.). Carrier-free Na^{125}I was obtained from New England Nuclear (Boston, MA, U.S.A.).

Enzyme assays

The hydrolyses of lactose and cellobiose were measured by the method of Dahlqvist (1964), with 28 mM substrate as the final concentration. Phlorizin hydrolase activity was determined by the method of Malathi & Crane (1968), with 2.5 mM as the final phlorizin concentration. *p*-Nitrophenyl β -D-galactosidase, *o*-nitrophenyl β -D-galactosidase and *o*-nitrophenyl β -D-fucosidase were assayed as described by Asp *et al.* (1969), with final concentrations of 13 mM of each substrate. All assays contained 1 mg of albumin/ml, buffered with 50 mM-sodium citrate/phosphate, pH 5.8 (buffer A), and were carried out at 37 °C for 60 min. Enzyme activities were linear with respect to time and protein concentrations. The molar absorption coefficients used for *p*-nitrophenol and *o*-nitrophenol were 1.75×10^4 and 4.16×10^3 respectively (Distler & Jourdan, 1973). An enzyme unit was defined as 1 μ mol of substrate hydrolysed/min at 37 °C. K_m and V_{max} were calculated by the Lineweaver–Burk (1934) plot. Lactose concentrations ranging from 1.5 to 60 mM were used for the kinetic measurements. The pH-dependence of lactase activity was studied in McIlvaine's (1921) buffers. Time-dependent inactivation of enzyme activities was studied with 15 and 45 μ g of enzyme/ml for lactase and phlorizin hydrolase respectively. This was done by measuring the residual enzyme activity after

each period of incubation at 45 °C. All enzyme-activity assays were performed in duplicate and the results averaged.

Preparation of lactase

Samples were obtained at autopsy 24–48 h after death and were used either immediately or stored at –80 °C for not more than 2 weeks before use. The samples were obtained from infants of Chinese extraction who were less than 2 years of age and were free from gastrointestinal diseases. Lactase was isolated from the brush-border membranes, prepared as described by Schmitz *et al.* (1973). In addition, the homogenization buffer contained 1 mM-phenylmethanesulphonyl fluoride and 10 mM-benzamidine hydrochloride to minimize hydrolysis by serine proteinases. Essentially whole intestine was homogenized and 10 mM-CaCl₂ was added to precipitate membranous structures other than those of the brush border, which were subsequently sedimented from the supernatant by centrifugation at 25000 g for 30 min. Solubilization of the brush-border membranes was effected with 0.2% Triton X-100/1 mM-EDTA in buffer A for 30 min at room temperature. A typical fractionation scheme is described for the purification of lactase: 20 mg of the solubilized membranes in a 10 ml solution was chromatographed at a flow rate of 10 ml/h on a concanavalin A–Sephacrose 4B column (23 cm × 1.2 cm) previously equilibrated with 2 mM-MgCl₂/2 mM-CaCl₂/1 mM-MnCl₂/0.1% Triton X-100/20 mM-glycylglycine buffer, pH 7.0. Concanavalin A was prepared from jack-bean meal as described by Olson & Liener (1967) and was bound to Sepharose 4B as described by Porath *et al.* (1967) in a ratio of 10 mg of protein/ml of packed gel, or the gel was purchased directly from Pharmacia. No difference was detected when these gel matrices were used in column chromatography. After the sample was loaded, the column was washed with 0.5 M-NaCl/0.01 M-sodium phosphate/0.1% Triton X-100, pH 6.0, until the A_{280} of the eluate was less than 0.02. The bound material was eluted by adding 0.25 M- α -methyl D-mannoside to the wash buffer. Pools of bound material were made from three runs of the concanavalin A–Sephacrose column and concentrated by ultrafiltration with an Amicon (Lexington, MA, U.S.A.) YM-10 membrane, before proceeding to a gel-filtration step. The concentrated material, containing about 7 mg of protein in 3 ml of solution, was filtered at 7 ml/h on Bio-Gel 1.5 m (1.2 cm × 73 cm), with 0.1 M-NaCl/0.01 M-sodium acetate/0.05% Triton X-100, pH 6.0. Fractions (1 ml) were collected and monitored for protein and by enzyme-activity assays. A second Bio-Gel 1.5 m column was used to rechromatograph the fractions containing enzyme activity from the previous step, under identical conditions. The Bio-Gel 1.5 m column was calibrated with Blue Dextran 2000, thyroglobulin (M_r 660000), ferritin (M_r 232000) from Pharmacia, and rabbit IgG (M_r 150000) and bovine serum albumin (M_r 68000) from Sigma. Finally chromatofocusing was used to purify the enzyme further. A column of PBE 94 (1 cm × 16.5 cm) was equilibrated with 25 mM-imidazole/HCl, pH 7.4, and, after the sample was loaded, was then washed with Polybuffer 74, pH 4.0, as recommended by the manufacturer. The sample used for chromatofocusing was a pool of lactase activity from the previous gel-filtration step, which contained about 1.2 units of enzyme in 10 ml,

and had been extensively dialysed against the imidazole buffer before use. Fractions (2 ml) were collected and the pH of each was read. (NH₄)₂SO₄ was added to each fraction (final concn. 95%, w/v) to precipitate the protein from interfering Polybuffer ions. The precipitates were then resuspended in 0.2 ml of buffer A and assayed for protein and lactase activity.

Immunization of rabbits and production of antibodies

The purified lactase (100 μ g) was mixed with 1.5 vol. of complete Freund's adjuvant and injected at multiple sites into two male New Zealand rabbits. The immunization and bleeding schedule was performed as described by Lau *et al.* (1979). IgG fractions were prepared as described by McGuigan (1968) and were used in all experiments involving the use of these antisera.

Preparation of anti-lactase-IgG–Sephacrose and immunoaffinity chromatography

Sephacrose 4B (5 ml) was treated with 10 ml of saturated CNBr, and the product was coupled to 27 mg of anti-lactase IgG as described by Porath *et al.* (1967). About 95% of the protein was found to be bound to the gel by measuring the difference in A_{280} of the protein solution before and after coupling. The Sepharose gel was loaded into a column (0.8 cm × 9.5 cm) and equilibrated with 2 mM-sodium phosphate/0.1 M-NaCl, pH 7.0. Next 2 ml of solubilized brush-border membranes containing about 20 mg of protein was loaded on the column, which was then washed with the equilibration buffer until the A_{280} reached 0.02. The column was eluted with 0.2 M-glycine/HCl, pH 2.5. The individual fractions eluted were immediately adjusted to pH 6 with 1 M-NaOH and assayed for enzyme activity.

Inhibition of lactase activity by anti-lactase IgG

Lactase, which was isolated either by the purification procedure (50 μ l of 0.05 mg/ml), or isolated by immunoaffinity chromatography (50 μ l of 0.2 mg/ml), was mixed with 20 μ l of anti-lactase IgG fractions, containing 2, 4, 6, 8 or 10 μ g of protein in buffer A to which 10 μ g of albumin had been added. The reactions were continued for 30 min at 37 °C, the precipitates cleared by centrifugation at 4 °C and the residual enzyme activity in the supernatants was assayed as described above. A control which contained non-immune rabbit IgG instead of the specific immunoglobulin was carried out in a similar manner, and its enzyme activity was taken as 100%. All activities were expressed as a fraction of the control. All the inhibition reactions were performed in duplicate and the results averaged.

Iodination and radioimmunoassay

Purified lactase (20 μ l of 1 mg/ml) was iodinated with 0.2 mCi of carrier-free Na¹²⁵I by using chloramine-T (Greenwood *et al.*, 1963). The final product contained 2.5×10^5 c.p.m./ μ g of protein and was used in a radioimmunoassay as described previously (Lau *et al.*, 1979); 50 μ l, containing 4000 c.p.m., was incubated overnight at 4 °C with 100 μ l of various antibody dilutions. Then 200 μ l of a second antibody (sheep anti-rabbit IgG) and 5 μ l of non-immune rabbit IgG (10 mg/ml) were added to the reaction mixtures and incubation was continued for another 18 h, after which time the immune precipitates were washed thoroughly and counted for radioactivity. This established the

Table 1. Purification of lactase from 50 g (wet wt.) of human small intestine

Procedure	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Recovery (%)
Homogenate	5600	168	0.03	1	100
Brush border	120	29	0.24	8	17
Concanavalin A eluate	4.6	12.6	2.76	92	7.5
1st Bio-Gel	0.12	2.2	17.9	598	1.3
2nd Bio-Gel	0.10	1.9	19.2	630	1.1
Chromatofocusing	0.07	1.5	23.0	755	0.9

antibody dilution to be used in the competition experiment. Samples (50 μ l) containing various concentrations of the competing antigens, lactase obtained either from the purification procedure or from the immunoaffinity column, were preincubated for 60 min with 100 μ l of anti-lactase dilution (0.3 μ g/ml). Then 50 μ l of 125 I-lactase (containing 4000 c.p.m.) was added to the mixtures and the incubation continued overnight. Thereafter the addition of the second antibody system to separate the bound radioactive lactase from the unlabelled lactase was carried out as described above. All reactions were carried out in duplicate and the results averaged.

Electrophoresis

Purified lactase was first radiolabelled with 125 I as described above, and run in SDS/5% -polyacrylamide-gel

Table 2. Mixed-substrate incubations with lactase that was eluted from concanavalin A-Sepharose

Incubations were carried out at 37 °C in 0.1 M-sodium acetate buffer, pH 6.0. Galactose was determined by the galactose dehydrogenase method of Doudoroff (1962) as described by Distler & Jourdan (1973), and liberated phloretin was calculated from glucose and galactose. The results are expressed as nmol of monosaccharide liberated/min per ml of protein. The concentrations of lactose and phlorizin used were 28 mM and 2.5 mM respectively. All assays were done in duplicate and the results were averaged. The individual determinations did not differ from each other by more than 10%.

	Galactose liberated	Glucose liberated	Phlorizin hydrolysed
Lactose	128.0	141.5	
Phlorizin		15.3	15.3
Lactose + phlorizin	62.6	79.8	17.2

electrophoresis as described by Laemmli (1970), under both reduced and non-reduced conditions. Radioactive gels were sliced into 2 mm pieces with a manual slicer from Hoefer (San Francisco, CA, U.S.A.), and the radioactivity was counted. M_r markers were employed during electrophoresis under non-reduced conditions. They were human plasminogen (91000), rabbit IgG (150000) and thyroglobulin (660000), and were stained by Coomassie Blue R.

Protein determination

Protein was monitored by the A_{280} during column chromatography, except in chromatofocusing, where protein was measured by the dye-binding method of Bradford (1976). The concentration of protein was quantified by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as standard.

RESULTS AND DISCUSSION

Human lactase had previously only been isolated by immunological methods (Skovjberg *et al.*, 1981; Potter *et al.*, 1985). The results of Skovjberg *et al.* (1981) could be difficult to reproduce, because the avidity of polyclonal antisera used is usually not under the control of the experimenter. The hybridoma technology used by Potter *et al.* (1985) also had its drawbacks, in finding a suitable screening procedure and by the rarity of a successful clone (Swallow *et al.*, 1985). In order to circumvent these problems, conventional column-chromatographic

Fig. 1. Biochemical characterization of the enzyme

(a) Heat-stability of phlorizin hydrolase activity (○) and lactase activity (●) as a function of time at 45 °C. The two enzyme activities were measured as described by Dahlqvist (1964) and Malathi & Crane (1968) respectively at the time points indicated, and were expressed as a fraction of the original activity at zero time. (b) Lactase activity as a function of pH. The purified protein was dialysed into McIlvaine (1921) buffers of different pH values. The lactase activity at each pH value was then determined and expressed as a fraction of the maximal activity (pH 6).

Table 3. Comparison of the enzyme activities toward different substrates

The purified enzyme was incubated with various concentrations (as indicated in parentheses) of different substrates for 60 min at 37 °C as described in the Materials and methods section. All assays were done in duplicate and the results were averaged. The individual determinations did not differ from each other by more than 10%.

Substrate	Specific activity	
	(μmol/min per mg) (% of lactase activity)	
Lactose (28 mM)	10.8	100
Cellulbiose (28 mM)	1.90	17.6
Phlorizin (2.5 mM)	1.62	15.0
<i>p</i> -Nitrophenyl β-D-glucoside (13 mM)	1.39	12.9
<i>p</i> -Nitrophenyl β-D-galactoside (13 mM)	1.09	9.9
<i>o</i> -Nitrophenyl β-D-galactoside (13 mM)	1.04	9.6
<i>o</i> -Nitrophenyl β-D-fucoside (13 mM)	1.50	13.9

techniques were used to isolate lactase from microvillar membranes. Only infant small intestines were used in this study, to avoid the decline in lactase which had been observed in all adult animals (Lifshitz, 1980) and particularly in man (Simoons *et al.*, 1977; Lebenthal *et al.*, 1975).

Human small intestine was reported to contain two β-galactosidases in the brush border, which have more neutral pH optimum (approx. 6), and a lysosomal enzyme, which has an acidic pH optimum (approx. 4) (Asp *et al.*, 1969; Gray & Santiago, 1969; Asp, 1971). Preliminary experiments were carried out to ascertain that the product after homogenization and Ca²⁺ precipitation was indeed brush-border membranes, by measuring the *p*-nitrophenyl β-galactosidase activity at pH 5.8 and 3.7. It was found that the ratio of the enzyme activity at pH 5.8 to that at 3.7 was about 0.9, starting from the crude homogenate through to the CaCl₂ precipitate. The supernatant after this step, when precipitated by centrifugation and then resuspended in buffer, shifted the pH-5.8/pH-3.7 activity ratio to 2.8. This observation was consistent with the notion that the isolated material was mainly brush-border membranes, where the 'neutral' β-galactosidase predominated. These membranes were solubilized and the insoluble membrane fragments removed by centrifugation. The enzyme activity at pH 5.8 of the resulting supernatant increased by 35%, whereas that at pH 3.7 decreased by 20%, suggesting that further 'neutral' β-galactosidase activity could be released by detergent. Although the use of *o*-nitrophenyl galactoside as a measure of lactase had been criticized previously (Furth & Robinson, 1965), the pH-5.8/pH-3.7 activity ratio appeared to give a useful indication of the progress of the isolation steps.

The supernatant was then fractionated on a concanavalin A-Sepharose column. A small amount of lactase activity came through the column, but a relatively large amount of lactase activity was bound by this matrix and was eluted by 0.25 M-α-methyl D-mannoside added to the wash buffer. To study the nature of these two enzyme

activities, fractions containing enzyme activity from three separate concanavalin A-Sepharose column runs were pooled and chromatographed on Bio-Gel 1.5 m. The enzyme activity that was not adsorbed by concanavalin A-Sepharose migrated close to the void volume of the column, whereas the concanavalin A-adsorbed activity migrated to a position corresponding to M_r approx. 380 000. The smaller enzyme, however, had a much higher specific activity, some 10–16 times that of the relatively larger enzyme. The smaller enzyme had an apparent K_m of 17 mM and a V_{max} of 17.5 μM/min (with 0.5 μg of protein), and the larger enzyme had an apparent K_m of 21 mM and a V_{max} of 5.6 μM/min (with 25 μg of protein), with lactose as substrate. This larger enzyme form could be a distinct high- M_r lactase, an aggregate of the M_r -380 000 species, or an aggregate of a contaminating lysosomal β-galactosidase, which had a lower pH optimum and therefore appeared less active when assayed at pH 6. High- M_r aggregates seem to be common among intestinal β-galactosidases. For instance, the rat 'acid' β-galactosidase changed its apparent M_r when gel-filtered under different buffer conditions (Asp, 1970). The human β-galactosidase with a low pH optimum also appeared as a M_r -156 000 or -600 000 species when studied by ultracentrifugation, or as a M_r -100 000 species during gel filtration, and the hetero-β-galactosidase formed monomers or dimers under different conditions (Asp, 1971; Gray & Santiago, 1969). The larger molecule separated from concanavalin A-Sepharose was not studied further, and only the smaller enzyme was studied further in the present work. Fractions from the Bio-Gel 1.5 m column containing the smaller-sized lactase activity were pooled and re-chromatographed on a second Bio-Gel 1.5 m column. The resulting fractions containing enzyme activity were again pooled and dialysed extensively against 25 mM-imidazole buffer, pH 7.4, and chromatofocused on a PBE 94 column. Both protein and enzyme activity were eluted at the same position, centred about pH 4.8. This agreed quite well with that of the rat enzyme, which was found to have a pI in the range 4.3–4.5 (Schlegel-Haueter *et al.*, 1972), or to comprise a doublet of bands, one at pI 4.2–4.3 and the other at 4.7–4.8 (Cousineau & Green, 1980). The purification procedure gave approx. 1% yield and about 750-fold purification starting from crude intestine homogenate, and is summarized in Table 1.

The properties of the purified protein were then studied. The enzyme was radiolabelled with ¹²⁵I and electrophoresed in SDS/5% polyacrylamide gel. A single radioactive peak was found under non-reduced or reduced conditions, and an apparent M_r of 170 000 was found for the protein by comparison with standard M_r markers under non-reduced conditions. This apparent M_r was slightly less than half of that found by gel-filtration. This discrepancy had been noted by Skovbjerg *et al.* (1981) and Potter *et al.* (1985).

Human lactase activity has been found to be associated with phlorizin hydrolase activity. Therefore the purified protein was studied for its heat-stability at 45 °C to investigate the behaviour of the protein with respect to these two activities. It appeared that lactase activity was more labile than phlorizin hydrolase activity for all time points tested (Fig. 1a). Mixed-substrate incubation experiments indicated that phlorizin inhibited lactase activity, but lactose did not inhibit phlorizin hydrolase activity (Table 2). These results agreed with

Fig. 2. Immunochemical characterization of the enzyme

(a) Immunoaffinity chromatography of brush-border fractions. Immunoglobulins of an antiserum raised against the purified enzyme were bound on Sepharose 4B, and solubilized brush-border membrane fragments were chromatographed on a column containing the gel. The column was washed until a constantly low A_{280} was reached, and then eluted with 0.2 M-glycine/HCl, pH 2.5, as indicated by the arrow. (b) Inhibition of lactase activity by anti-lactase IgG. Enzymes obtained from the purification procedure (●) or the immunoaffinity column (○) were incubated with various amounts of anti-lactase IgG for 30 min at 37 °C, and the residual activities were then assayed. The activities were expressed as percentages of that of a control which contained non-immune rabbit immunoglobulin instead of the specific antibodies.

previous findings from rat (Kraml *et al.*, 1972; Colombo *et al.*, 1973), human mucosal homogenate (Lorenz-Meyer *et al.*, 1972) and purified human lactase (Skovbjerg *et al.*, 1981), and supported the concept that these two enzyme activities were located on different catalytic sites and that there was addition inhibition of lactase by phlorizin. Furthermore, Skovbjerg *et al.* (1981) had also shown that lactase was competitively inhibited by phlorizin. Potter *et al.* (1985), however, presented data which argued for two equivalent subunits, each containing the same catalytic site for both substrates. A definitive answer as to whether the two catalytic sites belong to a single molecule or to two molecules linked non-covalently could be arrived at only if the subunits are separated and studied individually.

The purified enzyme showed maximal activity at pH 6.0 (Fig. 1b). When the enzyme was tested against different chromogenic substrates, it was found to have β -glucosidase activities, which varied between 13–18% of the lactase activity. The enzyme also hydrolysed other hetero- β -galactosides (Table 3).

Specific antibodies were then raised in two rabbits against the purified protein. Both rabbits were found to produce antisera which reacted with the purified enzyme in double immunodiffusion. The antiserum of the second bleeding from one of the rabbits was processed to yield IgG fractions, which were then bound to Sepharose 4B

via CNBr. The IgG-bound matrix was then used in immunoaffinity chromatography (Fig. 2a). Most of the loaded proteins passed through the column unimpeded, but a small protein peak which displayed lactase activity could be eluted by 0.2 M-glycine/HCl, pH 2.5. However, the specific activity of the enzyme prepared thus was considerably less than that prepared by conventional chromatographic steps (approx. 5 units/mg). This could be due to the acidic nature of the eluent used, which might have damaged the enzyme's catalytic apparatus. This is consistent with the findings that the enzyme prepared in this way was practically the same as that prepared by means of the purification procedure when only epitope structures and not enzyme activities were assayed. This was achieved by inhibition with anti-lactase antibodies (Fig. 2b) or in the radioimmunoassay for lactase.

A radioimmunoassay utilizing a double-antibody technique was established. The antibody population could immunoprecipitate approx. 75% of the radioactively labelled enzyme at a concentration of 0.5 mg/ml (Fig. 3a), but it was usually diluted to 0.3 μg/ml in order to increase the sensitivity of the assay, in which case 35–40% of the 125 I-lactase radioactivity could be precipitated by the antibodies. Enzymes obtained from the purification procedure and by the immunoaffinity column appeared to displace the radioactive antigen in

Fig. 3. Radioimmunoassay for lactase

(a) Binding of anti-lactase IgG to radioactive lactase obtained from the purification procedure. The enzyme was labelled with ¹²⁵I to 2.5×10^5 c.p.m./ μ g, and 50 μ l (containing 4000 c.p.m.) was incubated with 100 μ l of various dilutions of anti-lactase IgG. A second antibody system consisting of sheep anti-rabbit IgG and non-immune rabbit IgG were added to the mixtures on the second day and further incubated overnight. Then the immune precipitates were washed and counted for radioactivity. The percentage of radioactivity bound was then plotted against the anti-lactase IgG dilutions. (b) Competition between radioactive enzyme and unlabelled enzyme for anti-lactase. Lactase obtained from the purification procedure (●) or from the immunoaffinity column (○) was preincubated with various dilutions of anti-lactase IgG (0.3 μ g/ml). Then ¹²⁵I-lactase containing 4000 c.p.m. was added to the mixtures and the incubation continued overnight. The addition of the second antibody system, washing and counting were repeated as described above. Bound ¹²⁵I-lactase in the presence of competing antigen as a percentage of bound ¹²⁵I-lactase in the absence of competing antigen (% B/B₀) is plotted against the concentration of the antigen.

the same manner (Fig. 3b). The limit of detection in this assay was about 0.5 μ g of lactase/ml. This sensitivity should be as good as, or better than, the electroimmunoassay of Goda *et al.* (1984) for studying the dietary induction of rat lactase. The assay could facilitate studies

into the molecular basis of the changes of lactase amount in the human.

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