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Properties of lactase produced by enteropathogenic *Escherichia coli* from diarrhoeic children

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Summary

The quantity of lactase produced by enteropathogenic *Escherichia coli* (EPEC) was significantly higher ($P < 0.01$) than that produced by non-EPEC. The enzyme production was induced by lactose but repressed by glucose and galactose. The lactase from EPEC which was partially purified by ammonium sulphate precipitation and gel permeation chromatography had a molecular weight of 56 kD and apparent K_m of approximately 2.78 mM for lactose. The lactase exhibited optimum activity at pH 7.0 at 40°C and was stimulated by Mg^{2+}, Mn^{2+}, Na^+ and inhibited by Ba^{2+}, Ca^+, Cu^{2+}, EDTA, iodo acetic acid (IAA) and Hg^{2+} and U^{2+} ions. The higher production of lactase by EPEC may be linked to its pathogenic role in childhood diarrhoea.

Résumé

Nous avons constaté que *Escherichia coli* enteropathogénique (EPEC) produit une plus grande quantité ($P < 0.01$) de lactase que *Escherichia coli* non-pathogénique. Cette enzyme que provoque le lactose mais inhibe le glucose et le galactose a été partiellement purifiée par le précipitation suivie de dialyse et de chromatographie du gel de pénétration. Le poids moléculaire de la lactase EPEC partiellement purifiée était de l'ordre de 56 kD avec un K_m apparent de quoique 2.78 mM pour le lactose. La lactase a révélé une activité optimale de pH 7.0 à 40°C. Elle a été stimulée par Mg^{2+}, Mn^{2+}, Na^+ mais inhibée par Ba^{2+}, Ca^{2+}, Cu^{2+}, EDTA, IAA, Hg^{2+} et U^{2+}. La production plus grande de lactase par EPEC peut être associée à leur rôle pathogénique en diarrhées infantile.

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Introduction

Enteropathogenic *Escherichia coli* (EPEC) has been reported as an enteric pathogen to children under 2 years of age [1]. The pathogenesis of such *E. coli* strains in childhood diarrhoea is not clearly understood although the implication of extracellular byproducts such as toxins and enzymes by such organisms has been reported [2].

Invasion of the host cells by microorganisms has been reported to be enhanced by enzyme production [3]. Faecal *E. coli* was reported to produce maltase and lactase [4] and the maltase produced by EPEC was found to be more abundant than that produced by non-EPEC [5]. It was therefore decided to examine both EPEC and non-EPEC for lactase production quantitatively. The characterization of EPEC lactase was carried out similarly as this may indicate its role both in the pathogenesis of EPEC in childhood diarrhoea and other activities in the human enteric system.

Materials and methods

One hundred faecal samples from children aged 0–2 years with diarrhoea (60 females and 40 males) who were patients at the Ife University Teaching Hospital were examined for the presence of *E. coli* and EPEC. The faecal *E. coli* were identified by standard methods [4] while the EPEC strains were identified serologically

[1] using antisera prepared by Wellcome Laboratories (Beckenham, U.K.). Both EPEC and non-EPEC were tested for their ability to produce lactase quantitatively.

Preparation of inoculum, culture conditions, growth of organisms, preparation of bacterial cells for lactase production were as described by Olusanya [4].

Lactase was assayed by the glucose oxidase-O-dianisidine technique [6] using 0.5 mM lactose in 0.2 M citrate phosphate buffer (pH 7.0) as substrate. One unit of activity (U) is defined as the amount of enzyme liberating 1 μg glucose in 1 min.

Growth condition for catabolite repression and induction by lactase activity

The EPEC was cultured in the basal medium described by Olusanya [4]. The basal medium also contained lactose (0.3% w/v), lactose plus glucose (0.15 + 0.15% w/v) or lactose plus galactose (0.15 + 0.15% w/v). Basal medium in which either glucose (0.15%) or galactose (0.15%) were added to 0.15% lactose-containing medium after incubation for 4 h, were also set up. The induction experiments were set up as follows: EPEC was grown in the basal medium containing glucose (0.15%) or galactose (0.15%) at the onset of the experiment. After 4 h incubation, lactose (0.15%) was added to experimental flasks but not the control flasks. After a maximum incubation period of 10 h, all cultures were examined for the presence of lactase as described earlier.

Purification of enzyme

Ammonium sulphate precipitation. The crude enzyme preparation was precipitated with $(NH_4)_2SO_4$ between the limits of 40–90% saturation [7]. The precipitants were redissolved in 0.2 M citrate phosphate buffer (pH 7.0) for 18 h at 4°C. The dialysed filtrate was sterilized by membrane filtration before fractionation and assay procedures.

Gel permeation chromatography. A jacketed column was packed with Sephadex G-25 or G-100 to a height of 645 mm (25 mm diameter) and cooled to 4°C as described by Andrews [8]. The column was equilibrated and eluted on each occasion with 0.2 M citrate phosphate buffer (pH 7.0) containing 5 mM sodium azide.

Application of samples to the column, collection and protein measurement of column effluent (5 ml/fraction) from both G-25 and G-100 were as described by Olusanya [6]. Each fraction was assayed for lactase as described earlier.

Determination of the properties of lactase

The partially purified lactase was examined for its properties. The effects of pH, temperature, substrate concentration, cations and chemicals on the activities of the enzyme were determined by standard methods [6].

Results

All the 100 faecal samples from the children possessed *E. coli* but only 18 of them were EPEC. Both the faecal *E. coli* and EPEC produced lactase, however, the amount of lactase produced by EPEC was significantly ($P < 0.01$) higher than that produced by non-EPEC. The optimal production of lactase was similar to that reported earlier [4].

Repression and induction of lactase activity

Experiments were carried out in which lactose medium was supplemented with glucose and galactose after 0 and 4 h respectively (Fig. 1). A decrease in lactase activity was observed in the presence of each of the two carbon sources, repression being greatest when glucose or galactose was added to the lactose-containing medium at 0 h. This suggested that the inducible lactase of EPEC may be under catabolite repressional control of glucose and galactose.

Properties of EPEC lactase

Effect of pH and temperature. Lactase activity was found to be optimum at pH 7.0. The activity was better in the alkaline than in the acid range (Fig. 2). Lactase activity increased with increase in temperature, reaching its peak at 40°C (Fig. 2). The EPEC lactase was found to be sensitive to heat, losing all its activity within 1 min of heating at 60°C (Fig. 2). Both Ca^{2+} and K^+ were not able to reduce the effect

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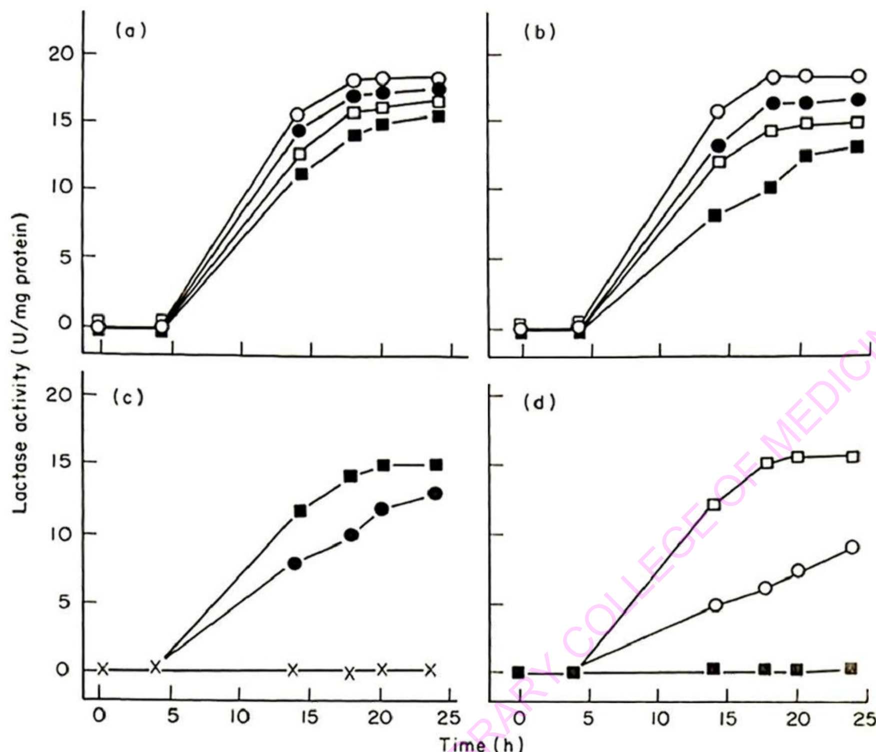

Fig. 1. Repression and induction of lactase activity of *E. coli* grown on single and mixed sources of carbon (a) 0.15% lactose plus 0.15% glucose (■); 0.15% lactose plus (after 4 h) 0.15% glucose (●); 0.15% lactose (□); 0.3% lactose (○). (b) 0.15% lactose plus 0.15% galactose (■); 0.15% lactose plus (after 4 h) 0.15% galactose (□); 0.15% lactose (●); 0.3% lactose (○). (c) 0.15% glucose plus 0.15% glucose plus 0.15% lactose (■); 0.15% glucose plus (after 4 h) 0.15% lactose (●); 0.15% or 0.3% glucose (×). (d) 0.15% galactose plus 0.15% lactose (□); 0.15% galactose plus (after 4 h) 0.15% lactose (○); 0.15% or 0.3% galactose (■).

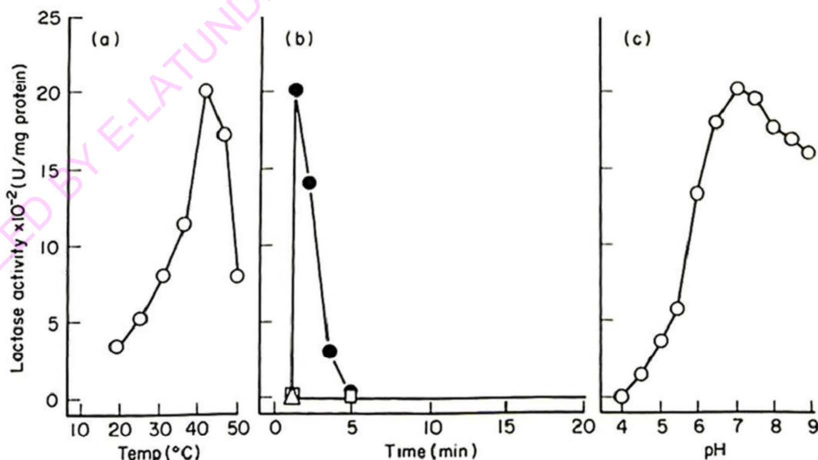

Fig. 2. Effect of (a) temperature, (b) heat (60°C) ((○) enzyme only, (△) enzyme plus Ca^{2+}, (●) enzyme plus albumin, and (□) enzyme plus K^{+}), and (c) pH on the activity of partially purified lactase (2nd ammonium sulphate fraction) obtained from cell homogenates of *E. coli*.

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of heat on the lactase. However, in the presence of albumin the effect of heat was reduced (Fig. 2).

The activity of EPEC lactase increased with an increase in the concentration of lactose, and was saturated at 17 mM. From the Lineweaver–Burk plot, the K_m for the hydrolysis of lactose was approximately 2.78 mM (Fig. 3).

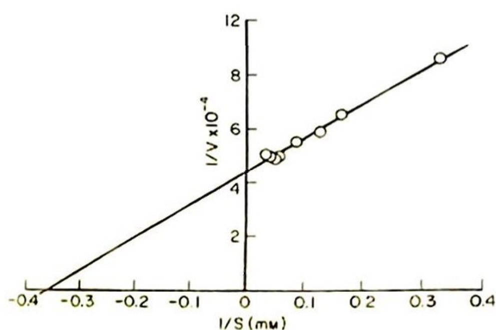

Fig. 3. Lineweaver–Burk plot for the hydrolysis of lactose by partially purified lactase (2nd ammonium sulphate fraction) obtained from cell homogenates of *E. coli* grown in lactose-peptone liquid medium.

Hydrolysis of different carbohydrates

The specificity of the enzyme was tested against a number of carbohydrates (Table 1). Lactase activity was detected only in the medium containing lactose and maltose, with optimum activity in lactose.

Table 1. Activity of partially purified lactase (2nd ammonium sulphate fraction) of *E. coli* on different carbohydrates

Substrates	Enzyme activity (U/mg protein)
Dulcitol	0.0
Inositol	0.0
Lactose	2000.33 ± 0.18*
Maltose	320.32 ± 3.13
Mannitol	0.0
Mannose	0.0
Starch	0.0
Sucrose	0.0

*Each value is the mean of three replicates ± standard error.

Effect of cations and chemicals

Magnesium, manganese and sodium (10–50 mM) ions were stimulatory to the EPEC lactase, while Ba^{2+}, Ca^{2+}, EDTA, Hg^{2+}, iodo acetic acid (IAA) and U^{2+} ions were inhibitory (Fig. 4).

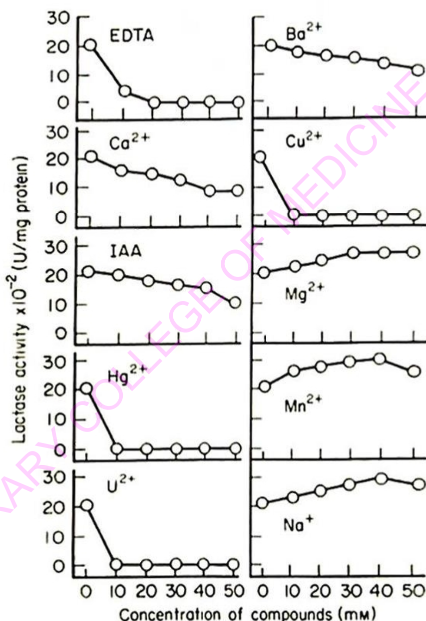

Fig. 4. Effect of various compounds and inorganic ions on the activity of partially purified lactase (2nd ammonium sulphate fraction) obtained from cell homogenates of *E. coli*.

Fractionation on Sephadex G-100

Fractionation of the concentrated culture filtrate on Sephadex G-100 led to four absorption peaks, designated Ba, Bb, Bc and Bd, with only peak Bb possessing lactase activity. The molecular weight of the active component (Bb) estimated from its elution volume was approximately 56 kD (Fig. 5), using myoglobin (MW 7800), egg albumin (MW 45,000), haemoglobin (MW 68,000) and glucose oxidase (MW 150,000) as standards.

Partial purification

The purification as outlined in Table 2 showed a final purification of 130-fold and specific activity of 2003 U/mg protein.

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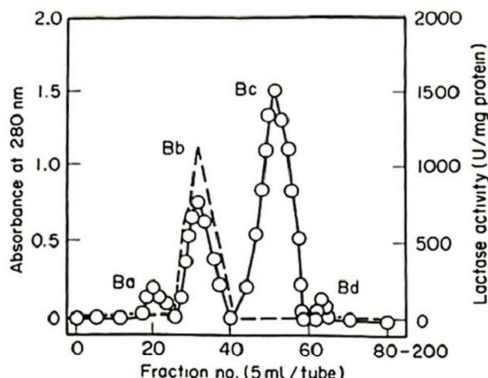

Fig. 5. Separation by gel filtration (Sephadex G-100) of proteins (fractions 12–20 previously separated by gel filtration on Sephadex G-25) and enzymic activity of the fractions towards lactose. (O———O) protein (E_{280}), (--------) lactase activity.

Discussion

It is significant to note that EPEC synthesized more lactase than non-EPEC. Enzyme production has been linked to the pathogenesis of bacteria [2], and this has been attributed to the enzymes enhancing the invasion of the host cells by the bacteria producing them [3]. It is therefore possible for the higher amount of lactase produced by EPEC to enhance their pathogenic role in childhood diarrhoea.

Glucose and galactose were reported to support the growth of faecal *E. coli* [4]. The repressive effect of these carbohydrates on the synthesis of EPEC lactase appeared to support the theory of catabolite repression. One of the mechanisms that has been linked to the theory is that it may prevent an organism from synthesizing an enzyme until it is needed [9].

The purification of 130-fold with specific activity of 2003 U/mg protein is high compared with other purified lactases previously reported [10]. The difference may be attributed to the combination of purification methods used by us. However, the molecular weight of 56 kD is much lower than the 135–280 kD reported [10]. The difference has been linked to the microbial origin of the lactase and the purification methods used [6].

The optimum activity of lactase at pH 7.0 and 40°C is similar to that reported for other bacterial lactases [11]. However, fungal lactases have been reported to be optimum at acid pH and at a higher temperature [12]. The specificity of lactase for lactose, is not surprising as this property had been used to differentiate lactase from β-galactosidase [13].

The optimum activity of EPEC lactase at pH 7.0 is similar to that in the intestine [14]. Coupled with the presence of lactose, an inducer of lactase [4], in the intestine this will enhance maximum production of lactase in the intestine. The maximum synthesis of lactase by such a strain of faecal *E. coli* in the intestine may thus be detrimental or beneficial to different individuals. The detrimental role occurs in the pathogenesis of EPEC in childhood diarrhoea, while its beneficial role could be in

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Table 2. Partial purification of lactase obtained from cell homogenate of *E. coli* grown in lactose-peptone liquid medium

Fraction	Total activity (U)	Total protein (mg)	Specific activity (U/mg protein)	Yield (%)	Purification (fold)
Crude extract	4150	270	15.4	100	1.0
Concentration (under vacuum at 30°C)	4025	249	16.2	97.0	1.1
Sephadex G-25	3737	37.3	100.2	90.0	6.5
1st $(NH_4)_2SO_4$ (40–90%) dialysed	3638	12.5	291.0	87.7	18.9
Sephadex G-100 (Peak Bb)	3627	3.2	1133.4	87.4	73.6
2nd $(NH_4)_2SO_4$ (40–90%) dialysed	2604	1.3	2003.1	62.7	130.1

helping the lactose intolerant people break down the lactose to the simple sugars which such individuals can assimilate.

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