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The Activity of Lactase (*Streptococcus thermophilus*) in Milk and Sweet Whey

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ABSTRACT

Activity of lactase (Streptococcus thermophilus) *in milk, sweet whey and milk salts was 33, 27 and 18%, respectively, of the activity in phosphate buffer containing a similar level of magnesium ions. The decrease is due primarily to the unfavorable ionic environment in milk products. Milk proteins activated the enzyme only slightly in buffer (6–14%), but seemed to ameliorate the inhibiting effect of the ionic environment in milk and whey. Activity in raw milk was 6–8% less than in the same milk heated to 63 or 85°C for 30 min.*

INTRODUCTION

Streptococcus thermophilus, which is widely used as a starter organism for yoghurt manufacture, is a safe and promising source of lactase (β-galactosidase; EC 3.2.1.23). The enzyme from this source has a neutral pH optimum, has a temperature optimum in buffer of 55 °C and is more heat stable than the neutral-pH lactases from yeasts which are available commercially (Greenberg & Mahoney, 1982). The enzyme has been purified to homogeneity (Ramana Rao & Dutta, 1981) and its activity on the synthetic substrate *o*-nitrophenyl β-D-galactopyranoside has been well characterised (Somkati & Steinberg, 1979; Greenberg & Mahoney, 1982). However, there is essentially no information regarding its activity

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in dairy products where the behavior may be quite different from that in model systems. The purpose of this study was to examine the activity of the enzyme in milk and sweet whey, and to ascertain some of the factors which determine the activity.

MATERIALS AND METHODS

Materials

Lactose and ovalbumin were from Sigma Chemical Co., St. Louis, MO. Sweet whey powder was a gift from Rich Dairy Products, Scituate, MA. Non-fat dry milk powder was from Yankee Milk Cooperative, Springfield, MA. Ultrafiltration membranes were from Amicon Corp., Lexington, MA. Casein (acid) was from US Biochemical, Cleveland, OH. All other chemicals were reagent grade or better.

Methods

Preparation of milk and protein fractions

Skim milk (10% solids) and sweet whey (7% solids) were prepared by reconstituting milk and whey powders with water containing 0·02% NaN_3, and stored at 4 °C; the pH values of the products were 6·6 and 5·8, respectively. Raw milk was skimmed to remove the fat by centrifugation and samples were heated at 63 and 85 °C for 30 min and then cooled at once to 0 °C; later that day they were used as substrates for lactase activity determinations.

Milk salt was prepared by ultrafiltration of milk at 4 °C using a YM-5 membrane with a nominal cut-off of 5000 daltons. It contained approximately 0·5 mg protein/peptide/ml as determined by a modification of the Lowry method (Toennies & Feng, 1965) and had a pH of 6·6 at 20 °C.

Casein (25 mg/ml), ovalbumin (5 mg/ml) and reduced glutathione (0·2 mg/ml) were dissolved directly in buffer A (0·1 M potassium phosphate, pH 6·6, 3·2 mM MgCl, 0·02% NaN_3) containing 5% lactose. The whey protein fraction was prepared by dialysing whey twice against buffer A containing 5% lactose, and containing 5·0 mg protein/ml.

Enzyme activity assays

The lactase from *S. thermophilus* was a partially purified preparation, free of protease activity, prepared as described by Greenberg & Mahoney (1982) with a specific activity of about 130 ONPG units/mg protein.

Initial rates of lactose hydrolysis were determined by adding 0·1 ml enzyme (7 ONPG units/ml) to 4 ml substrate solution at 37 °C. The reaction was stopped after 10 min by adding 0·1 ml 4 N HCl and the mixture deproteinised by adding 1 ml of 10% $ZnSO_4 . 7H_2O$. The resulting mixture was then neutralised by adding 0·16 ml 4 N NaOH and centrifuged for 15 min at 10 000*g*. The clear supernatant was analysed for glucose using the Glucostat procedure (Worthington, 1972).

Activity in sweet whey was determined at its original pH (5·8) and also at pH 6·6. To compensate for the potassium ions added as KOH raising the pH, an equal amount of potassium was added as KCl (43·3 mg KCl per 100 ml) to some samples of sweet whey and also to milk.

RESULTS AND DISCUSSION

Enzyme activity in milk and whey

The activity of the enzyme in various milk and whey systems is shown in Table 1. The activity in milk was less than one-third of that in potassium phosphate buffer containing the same level of magnesium, while the activity in sweet whey (pH 5·8) was 17% less than in milk.

The pH optimum of this enzyme on 5% lactose is 6·5–6·6 (Greenberg, 1982). Raising the pH of the whey to that of milk (pH 6·6) increased the activity by 16%. This was due in part to the added potassium ions (as KOH), as can be seen from the effect of adding an equivalent amount of KCl to the whey. Greenberg & Mahoney (1982) reported that potassium ions, in the range 0·1–100 mM, activated the enzyme in Tris buffer; these data show that the same effect occurs in milk systems. Lactase activity in whey at pH 6·6 was still 12% lower than in milk containing an equal amount of added potassium. This implies that the higher activity in milk is connected in some way with the presence of the casein component.

Although the pH of sweet whey is not optimal for the enzyme it may not be worthwhile neutralising the whey with alkali prior to processing. The activity would rise by 16% but against that must be set both the cost of the

TABLE 1
Activity of Lactase in Milk, Sweet Whey, Milk Salts and Buffer Systems

Substrate	*Activity (μmol glucose/min/ml)*	*Relative percentage*
5% Lactose in buffer A[a]	0·752	100
Milk[a]	0·249	33·1
Milk + KCl[a]	0·274	36·4
Sweet whey, pH 5·8	0·207	27·5
Sweet whey, pH 5·8 + KCl[b]	0·221	29·3
Sweet whey, pH 6·6	0·241	32·0
Milk salts[a]	0·136	18·1
Milk salts, pH 5·8	0·079	10·5
Milk salts + casein[a]	0·269	35·8

[a] pH 6·6.
[b] 5·8 mM.

KOH and the increased salt content which might restrict some applications of the hydrolysed-lactose product.

The activity of the enzyme in milk salts was 45% less than in milk and 62% less than in sweet whey, when compared at equivalent pH values. In this respect, this lactase behaves differently than the yeast lactase from *Kluyveromyces fragilis* whose activity in milk and whey ultrafiltrates was the same as in the parent milk and whey (Mahoney & Adamchuk, 1980). Compared to buffer A, milk salts contain 18 mM sodium, 9 mM soluble calcium and much less potassium (40 mM compared to 146 mM in the buffer) (Jenness & Koops, 1962) all of which would serve to depress the activity of the enzyme (Greenberg & Mahoney, 1982). They also contain 9 mM citrate, and when this was added to buffer A, lactase activity fell by 16%, presumably because the citrate complexed with the magnesium ions which are activators (Greenberg & Mahoney, 1982).

Effect of milk proteins on lactase activity

Since the activity in milk and whey is much higher than in milk salts, it seems that the milk proteins must have some role. The effect of various milk protein fractions, of ovalbumin (a non-milk protein) and of reduced glutathione, on enzyme activity in buffer is shown in Table 2. Both casein

TABLE 2
Effect of Milk Proteins, Ovalbumin and Glutathione on Lactase Activity

Substrate	*Activity* (*μmol glucose/min/ml*)	*Relative percentage*
5% Lactose in buffer A	0·884	100
Casein[a]	0·936	106
Whey protein[a]	1·01	114
Ovalbumin[a]	0·978	111
Reduced glutathione[a]	0·984	111

[a] In buffer A with 5% lactose.

and the whey proteins were slightly activating (6–14%) but so was ovalbumin, which suggests that the effect is not specific for milk proteins. It is possible that the enzyme benefits from the better reducing environment since peptide-bound sulfydryl groups, in the form of reduced glutathione, activated the enzyme about as much as any of the milk proteins.

Although the milk proteins have very little effect on activity in buffer, their presence in milk and whey caused a large increase in activity compared to the salts. Adding casein (25 mg/ml) to milk salts caused the activity to rise by 90% (Table 1). It seems likely that the proteins and milk salts interact in some fashion so as to produce a more favorable ionic environment for the enzyme. How this occurs is not clear, but it could be that the proteins interact with inhibiting ions such as calcium and thereby raise lactase activity.

Effect of heating milk on activity

Preheating the raw milk had very little effect on enzyme activity. When the milk was heated to 63 and 85°C for 30 min, the initial rate of hydrolysis was 8 and 6% higher, respectively, than when using unheated milk. Wendorff (1969), using lactase from *Saccharomyces fragilis*, reported a 15% increase when raw milk was preheated at 74°C and Wendorff *et al.* (1970) reported an activity increase of over 90% when skim-milk concentrate was heated to 85°C. They further suggested that milk contained a thermolabile lactase inhibitor (Wendorff *et al.*, 1971) but our results do not support this concept.

CONCLUSIONS

The activity of this lactase in milk and whey is much lower than in model (buffer) systems primarily because the ionic environment is less favorable. The inhibiting effect of the milk salts environment is ameliorated to some extent by the presence of the milk proteins, although the latter have very little effect by themselves in a buffer system. Lactase activity in pasteurised milk was slightly higher than in raw milk but thermal processing does not appear to confer any significant advantage.

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