THE EFFECT OF TEMPERATURE ON A REACTION CATALYSED BY LACTOSE SYNTHETASE *A* PROTEIN

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1. Introduction

The *A* protein of lactose synthetase (EC 2.4.1.22) is a galactosyl transferase which catalyses both the synthesis of lactose from UDPgalactose and glucose and the synthesis of *N*-acetyl-lactosamine from UDP-galactose and NAG† [1]. Addition of α-lactalbumin lowers the K_m for both glucose and NAG and greatly enhances the first reaction, but it may either enhance or inhibit the second reaction. It activates the enzyme from cow's milk at low NAG concentrations and inhibits it at high concentrations [1–3], whereas it inhibits the enzyme from human milk at all NAG concentrations [4]. These differences have now been reconciled and explained by taking into account the effect of temperature on the reaction.

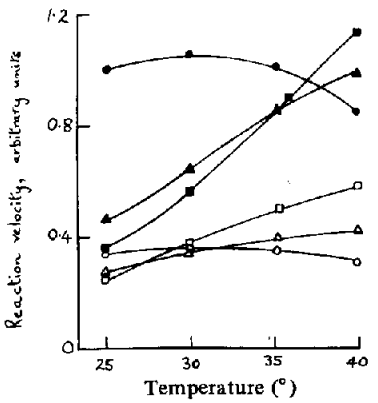

Fig. 1. Effect of temperature on galactosyl transfer to NAG catalysed by lactose synthetase *A* protein in the absence and presence of α-lactalbumin. NAG concentrations: 1 mM, closed symbols; 0.2 mM, open symbols. α-Lactalbumin concentration: ● 0, none; ▲ △, 200 μg/ml; ■ □, 500 μg/ml.

2. Materials and methods

Lactose synthetase *A* protein was isolated from human milk as described by Andrews [5]. The procedure described by Barman [6] for cow's milk was applied to human milk to obtain the α-lactalbumin.

Galactosyl transfer from UDPgalactose to NAG was determined by assaying spectrophotometrically the amount of UDP formed from the UDPgalactose [7]. The assay mixture contained, in a final volume of 1 ml, 50 μmoles of Tris-HCl with the pH adjusted to give pH 7.5 at the assay temperature, 4 μmoles of $MnCl_2$, 1 μmole of phosphoenolpyruvate, 0.1 μmole of ATP, 0.2 μmole of NADH, 0.4 μmole of UDPgalactose, 0.5 mg of crude pyruvate kinase (type 1, Sigma London Chemical Co., Ltd., London) and lactose synthetase *A* protein, NAG and α-lactalbumin as required. The assay temperatures were measured in the cell compartment of the spectrophotometer.

3. Results

The effect of temperature on the activity of *A* protein in the absence and presence of α-lactalbumin

† *Abbreviations*
NAG: *N*-acetyl-D-glucosamine.

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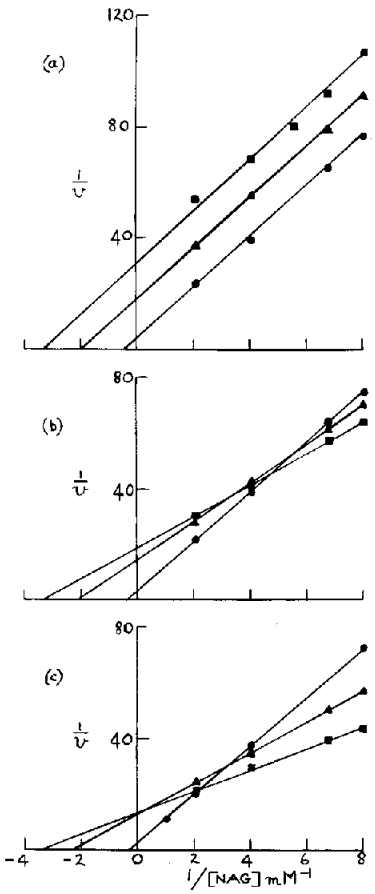

Fig. 2. Reciprocal plots of reaction velocity against NAG concentration for galactosyl transfer to NAG catalysed by lactose synthetase *A* protein in the absence and presence of α-lactalbumin at different temperatures (a) 25°. (b) 30°. (c) 37°. α-Lactalbumin concentrations: •, none; ▲, 200 μg/ml; ■, 500 μg/ml. Initial velocity *v* is expressed as absorbance change/min.

at two low NAG concentrations is shown in fig. 1. In the absence of α-lactalbumin the temperature for maximum activity was about 30° at both NAG concentrations and in each case the activities at 37° were about the same as those at 25°. The lower activities at 40° were evidently not due to irreversible enzyme inactivation since enzyme incubated in assay mixtures at 40° for 15 min and then assayed at 30° still showed the same activity as enzyme preincubated and assayed at 30°. The temperature for maximum activity was raised in the presence of α-lactalbumin to at least 40° under the conditions

Table 1
Effect of temperature on K_m for NAG and V_{max} of galactosyl transferase at different α-lactalbumin concentrations.

	K_m (mM)			V_{max} (arbitrary units)		
	α-Lactalbumin concentration (μg/ml)					
Temperature (°)	0	200	500	0	200	500
25	3.0	0.50	0.31	3.3	0.55	0.35
30	3.1	0.48	0.31	3.6	0.69	0.54
37	3.6	0.44	0.28	3.3	0.77	0.72

investigated, so that at the higher temperatures enzyme activity was greater in the presence of α-lactalbumin than in its absence.

Since the effect of temperature on the enzyme-catalysed reaction varied with α-lactalbumin concentration, the pattern of reciprocal plots of reaction velocity against NAG concentration at various α-lactalbumin concentrations also varied with temperature (fig. 2). The pattern at 25° was one of parallel straight lines whereas at 30° and 37° the lines crossed to the right of the velocity axis, the crossing points being nearer the axis at the higher temperature. The effect of temperature on V_{max} and the K_m for NAG in the absence and presence of α-lactalbumin is recorded in table 1.

4. Discussion

The galactosyl transferase from human milk resembles several other glycosyl transferases in having maximum activity at a lower temperature than is usual for enzymes. For example, maximum activity at 24° was reported for a galactosyl transferase from Ehrlich ascites tumour cell membranes [8] and at 22°, 28° and 37° for particle-bound galactosyl, *N*-acetylglucosaminyl and mannosyl transferases, respectively, from epithelial cells of rat small intestine [9]. A sialyl transferase found in soluble form in human serum and in membrane-bound form in the erythrocytes had maximum activity at 37° in both instances [10], suggesting that the temperature optimum is a characteristic of the enzyme itself rather than of the form in which it occurs. It is notable, therefore,

that formation of a complex with α-lactalbumin considerably raises the temperature optimum of galactosyl transferase from human milk.

The various observations on the effect of α-lactalbumin on the activity of galactosyl transferase from human [4] and cow's milk [1–3] with NAG as the acceptor substrate are illustrated by our present results with enzyme from the one source. At 25°, as already reported [4], α-lactalbumin inhibits the human milk enzyme at all NAG concentrations with the kinetic characteristics of an uncompetitive inhibitor (fig. 2a). On the other hand, at higher temperatures and low NAG concentrations the activity of the human milk enzyme, like that of the cow's milk enzyme at 30° [3] and 37° [1,2], is greater in the presence than in the absence of α-lactalbumin, with the difference being more pronounced at 37° than at 30° (figs. 2b, 2c). However, the activating effect of α-lactalbumin shown in our results is considerably less than that observed at 37° with the cow's milk enzyme [2].

Morrison and Ebner [3] propose that reactions catalysed by the galactosyl transferase from milk can proceed via two alternate pathways, the one catalysed by the transferase alone and the other, with a lower V_{max}, catalysed by a transferase-α-lactalbumin complex. Complex formation with α-lactalbumin also lowers the K_m of the enzyme for NAG and these authors suggest that the activating effect of α-lactalbumin on the reaction at low NAG concentrations is caused by the lower K_m increasing the effective concentration of NAG as regards the slower pathway, to the extent that the resultant greater proportion of the reaction proceeding along this pathway more than compensates for its lower V_{max}. This is evidently not the explanation, since the effect of α-lactalbumin on K_m is very much the same at 25° as at higher temperatures (table 1) yet α-lactalbumin is not an activator at 25°. Our results indicate instead that the greater transferase activity in the presence of α-lactalbumin at the higher temperatures arises because V_{max} for the reaction catalysed by enzyme alone fails to increase with temperature in the usual way. When associated with α-lactalbumin the enzyme shows a more normal response to temperature, so the activating effect of α-lactalbumin is more apparent than real.

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