THE ROLE OF α-LACTALBUMIN IN LACTOSE SYNTHETASE

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The A protein of lactose synthetase can catalyze lactose formation from UDP-galactose and glucose even in the absence of α-lactalbumin but only poorly because of a very high K_m for glucose. α-Lactalbumin decreases the K_m for glucose as well as that for N-acetylglucosamine (NAG). Depending only on the substrate concentration α-lactalbumin can stimulate disaccharide formation or inhibit this process both with NAG and with glucose. The affinities of the two sugars are such that under normal assay conditions, in the presence of α-lactalbumin, the concentration of glucose is optimal for lactose synthesis whereas that of NAG is inhibitory.

Lactose synthetase, an enzyme present in mammary glands (1) and in milk (2) is responsible for the last step in lactose biosynthesis according to the following reaction:

$$\text{UDP-galactose} + \text{glucose} \xrightarrow{Mn^{++}} \text{lactose} + \text{UDP} \qquad (1)$$

Brodbeck and Ebner showed that the enzyme responsible for reaction (1) is made up of two protein components (3). One of these proteins is the well known milk protein, α-lactalbumin, and is, by itself, inactive (4). The other protein, designated the A protein, was reported to be incapable of catalyzing reaction (1) in the absence of α-lactalbumin but can promote the efficient transfer of the galactosyl moiety of UDP-galactose to other acceptors such as N-acetylglucosamine (NAG) (5). In this latter case, the product of the reaction is N-acetyllactosamine, and α-lactalbumin was found to act as an inhibitor instead of a promoter as when glucose is the acceptor (5).

We have recently obtained a preparation of A protein from bovine milk which is free of contaminating proteins (6). In this communication we describe the results of some kinetic experiments using this preparation which are aimed at

clarifying the mechanism by which α-lactalbumin exerts its function as a "specifier protein" (5). Our results show that (a) the A protein can catalyze reaction (1) but only poorly because of a very high K_m for glucose; (b) α-lactalbumin decreases the K_m for glucose as well as that for NAG; and (c) α-lactalbumin inhibits the overall reaction at relatively high NAG concentrations and also at high glucose concentrations. Thus, at the concentrations of glucose which are ordinarily used in assays or found *in vivo*, α-lactalbumin greatly stimulates lactose synthesis. At similar concentrations of NAG, α-lactalbumin acts as a potent inhibitor. This mechanism of α-lactalbumin action relies on a poorer affinity of lactose synthetase for glucose than for NAG, both as substrates and as inhibitors.

MATERIALS AND METHODS

The A protein was prepared from raw bovine skim milk obtained from a local dairy by procedures modified (6) from those of Brodbeck and Ebner (3) and Brew *et al.* (5). The enzyme was shown to be free of contaminating proteins by sedimentation velocity and equilibrium, disc gel electrophoresis, and chromatographic techniques. The specific activity of the pure A protein is 4.5 units/A_{280}. One unit of enzyme catalyzes the formation of 1 μmole of lactose/minute in the standard assay.

Enzyme assays were performed at 37° using the method of Brew *et al.* (5) with only minor modifications. The standard assay mixture contained $MnCl_2$, 4 μmoles; Tris HCl, pH 7.4, 5 μmoles; glucose (or NAG), 2 μmoles; UDP-^{14}C-galactose, 0.1 μmole (50,000 cpm); UTP, 0.5 μmole; α-lactalbumin, 200 μg; and enzyme, 1-5 x 10^{-4} units in a total volume of 100 μl. When assays were performed in the absence of α-lactalbumin, it was necessary to add bovine serum albumin (10 μg) in order to maintain linear enzyme responses.

Bovine α-lactalbumin was a 3 times crystallized product obtained from the Gallard-Schlessinger Company. UDP-^{14}C-galactose was obtained from the New England Nuclear Company and diluted for use to a specific activity of 0.3 μC/μmole with the nonradioactive compound obtained from Calbiochem.

Paper chromatographic separation of lactose and N-acetyllactosamine was performed using as solvent the upper phase of pyridine, ethyl acetate, water (1 : 2 : 2) at room temperature. The spots were located by counting the chromatograms on a strip counter.

RESULTS

We have found that α-lactalbumin (1 mg/ml, a 10^4 molar excess over A protein) decreases the K_m of the A protein from approximately 1 M in its absence to 5.2×10^{-3} M in its presence. Intermediate concentrations of α-lactalbumin yield intermediate K_m values as is shown by the family of lines of Fig. 1. The figure also shows that the theoretical (extrapolated) maximal velocity of the reaction is unaffected by the presence of α-lactalbumin. In contrast to this theoretical expectation it is observed that at very high glucose concentrations,

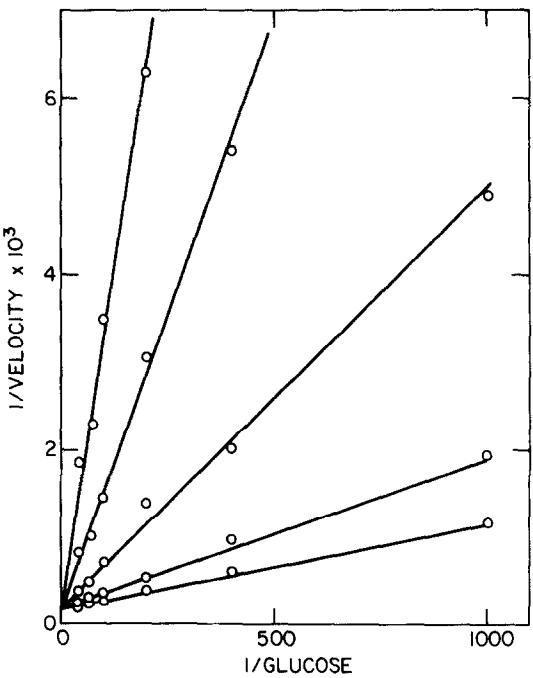

FIG. 1. Lineweaver-Burk plot of the initial rate of lactose synthesis as a function of glucose concentration. The lines correspond to (from top to bottom) 50, 100, 200, 500, and 1,000 μg α-lactalbumin/ml. The K_m values found are 390, 72, 25, 9, and 5, all $\times 10^{-3}$ M.

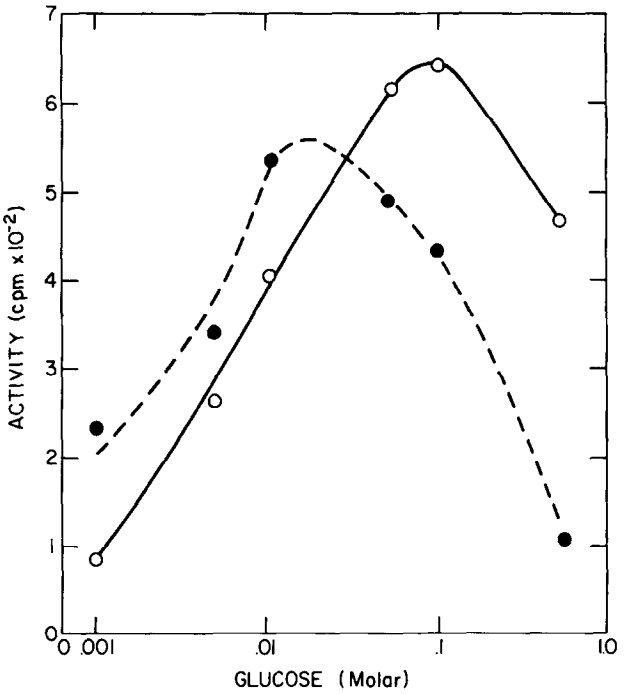

FIG. 2. Inhibition of lactose synthetase by α-lactalbumin at high glucose concentrations. Note the logarithmic abscissa. α-Lactalbumin is at 500 (o) and 2,000 (•) μg/ml.

α-lactalbumin inhibits lactose synthesis (Fig. 2). Thus, the nature of the effect of α-lactalbumin in the lactose synthetase complex depends upon the glucose concentrations; at glucose levels of 0.01 M and below the effect is to stimulate the reaction by lowering the K_m, whereas at glucose levels of 0.1 M and above, the effect is to promote substrate inhibition.

Exactly the same picture emerges when N-acetyllactosamine synthesis is studied by using NAG rather than glucose as substrate. Here, too, at low NAG concentrations, α-lactalbumin stimulates the reaction by decreasing the K_m for NAG (Fig. 3). Also in this case, at high NAG concentrations, α-lactalbumin exerts an inhibitory effect (Fig. 4). The only difference observed in the lactalbumin effect in the case of the two sugars is a quantitative one. Thus, whereas glucose utilization is stimulated up to glucose concentrations of 0.01 M or so, stimulation by α-lactalbumin of NAG utilization is seen only up to

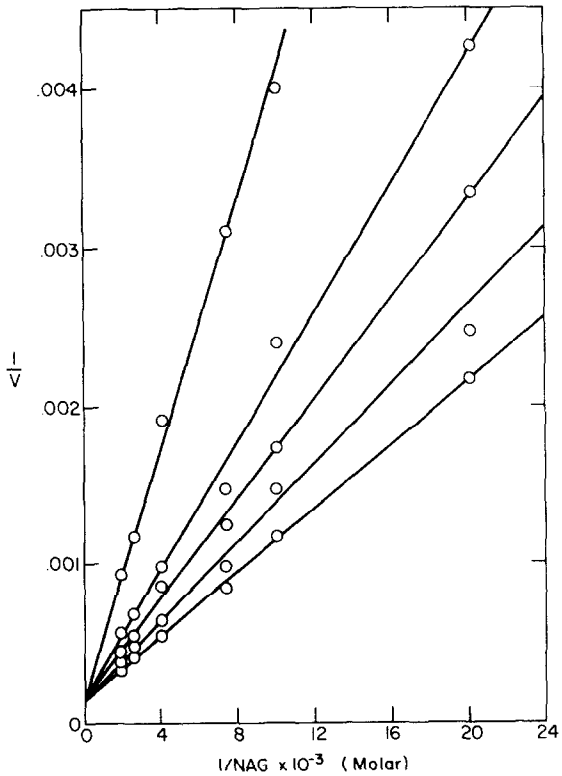

FIG. 3. Lineweaver-Burk plot of the initial rate of N-acetyllactosamine synthesis as a function of NAG concentration. (Only very low NAG levels were used here.) The lines correspond to (from top to bottom) 0, 50, 200, 500, and 1,000 μg α-lactalbumin/ml. The K_m values found are 3.6, 1.8, 1.4, 1.1, and 0.9, all x 10^{-3} M.

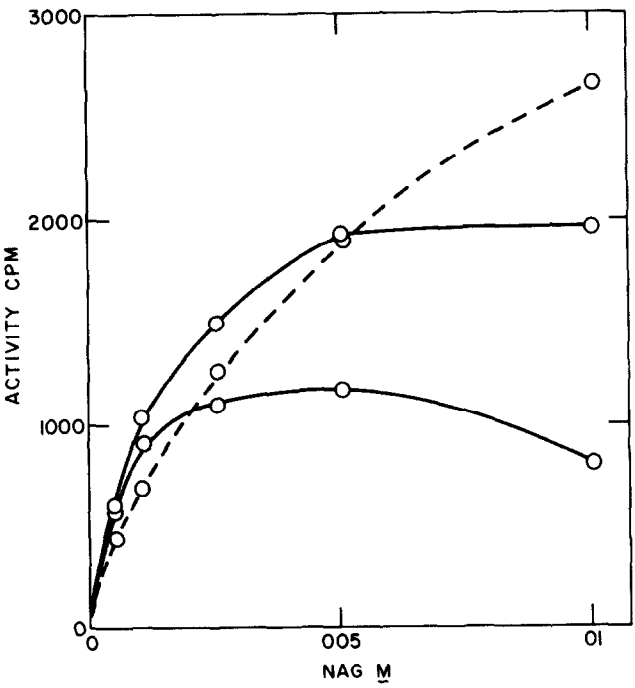

FIG. 4. Velocity of N-acetyllactosamine synthesis by A protein as a function of NAG concentration. The α-lactalbumin concentrations are 0 (dashed line), 200 (upper solid line), and 1,000 (lower solid line) μg/ml.

concentrations of about 0.001 M. Similarly, inhibition by substrate in the presence of α-lactalbumin occurs at much lower concentrations of NAG (~ 0.01 M) than of glucose (~ 0.1 M). It can also be seen in Figs. 2 and 4 that the extent of the inhibition seen at high glucose or NAG concentrations depends on the amount of α-lactalbumin present as well as on the sugar concentration. However, we have observed substrate inhibition even in the absence of α-lactalbumin at very high concentrations of NAG (0.1 M) and of glucose (2 M).

An alternative approach to some of these questions is to examine the effects of NAG and of glucose on each other's reaction. Fig. 5 shows that glucose has no inhibitory effect on the NAG reaction (measured in the absence of α-lactal-

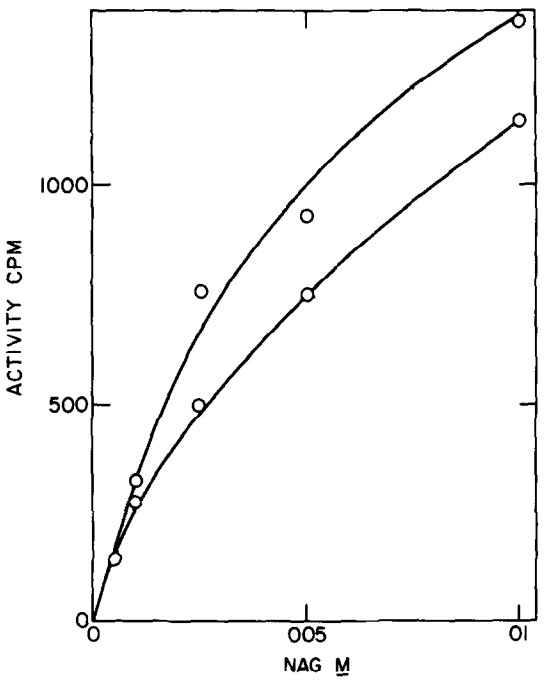

FIG. 5. The effect of 0.01 M glucose on the rate of N-acetyllactosamine synthesis by A protein.

bumin), although it may stimulate the overall reaction slightly. On the other hand, NAG is a potent inhibitor of glucose utilization (Fig. 6) measured in the presence of α-lactalbumin (1 mg/ml). The data of Fig. 6 show that 50% inhibition of glucose utilization by NAG occurs at an NAG concentration of about 5 x

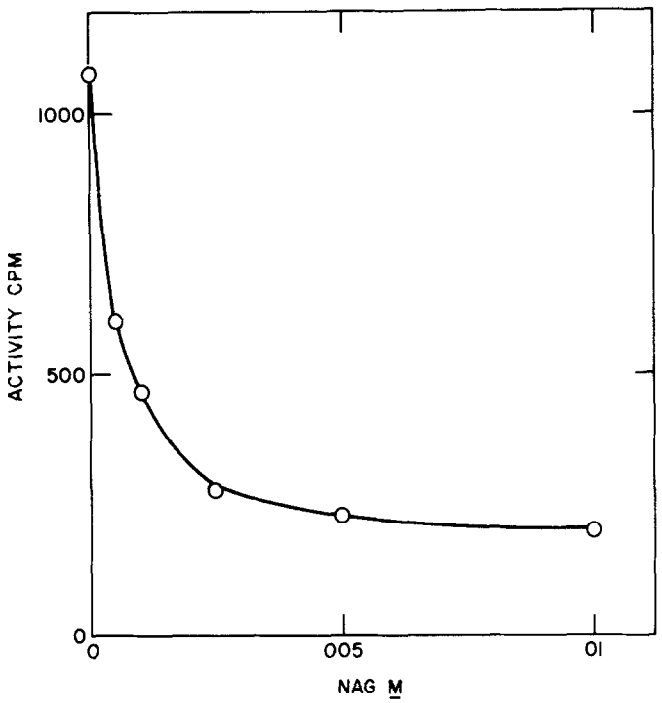

FIG. 6. The effect of NAG on glucose utilization by lactose synthetase.

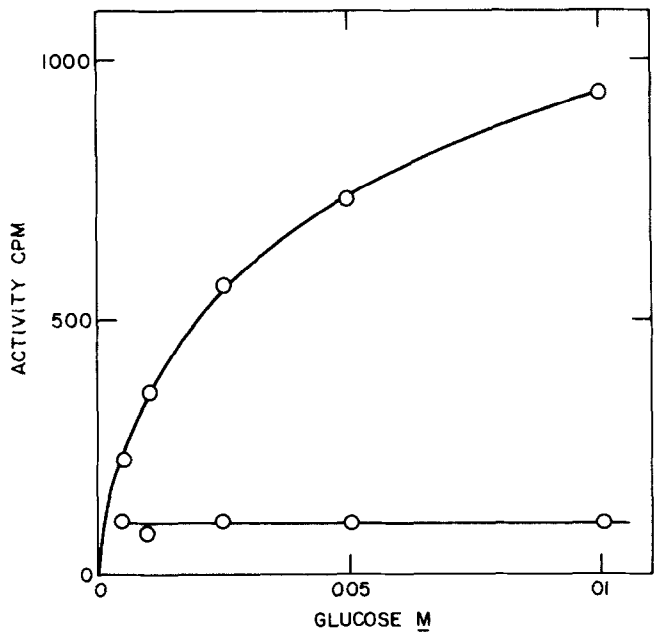

FIG. 7. The effect of NAG on glucose utilization by lactose synthetase. Upper curve, no NAG; lower curve, 0.01 M NAG.

10^{-4} M. This is an order of magnitude lower than the K_m for NAG in the absence of α-lactalbumin but is about equal to the K_m for NAG in the presence of 1 mg/ml α-lactalbumin. It is therefore probable that the K_m for NAG is a true measure of its binding affinity for the lactose synthetase complex. Fig. 6 also shows that the inhibition by NAG does not approach completion but rather a state in which 10% to 20% of the maximal activity remains. This is seen more dramatically in Fig. 7 in which a constant, and high (0.01 M), concentration of NAG is used at varying glucose concentrations. At all levels of glucose tested, the rate has been reduced to the same value of about 10% of the maximal rate. When a chromatographic analysis was performed of the products of the reaction, the results shown in Table I are obtained. These results show that NAG is used in preference to glucose as substrate even in the presence of α-lactalbumin so long as glucose is not present in large excess over NAG. They are therefore in accord with the relative K_m values determined above and are consistent with a competition between the two substrates for the enzyme.

TABLE I

Products of Lactose Synthetase Action with Mixed Substrates

Substrates	α-Lactalbumin	Products	
		Lactose	N-Acetyllactosamine
0.01 M	*1 mg/ml*	*% of Total*	
Glucose	+	100	0
NAG	0	0	100
Glucose + low NAG (0.0005 M)	+	50	50
Glucose + high NAG (0.01 M)	+	< 5	> 95

DISCUSSION

Our experiments have shown that α-lactalbumin serves to promote the binding of both glucose and NAG to lactose synthetase. α-Lactalbumin's physiological role as a protein necessary for lactose synthesis is in accord with this simple

function since the K_m of the isolated A protein for glucose is inordinately high [see also (7) and (8)].

The second effect of α-lactalbumin in the lactose synthetase system is to cause the appearance of substrate inhibition at concentrations of glucose or NAG approximately 10 times their K_m values. Here, too, no distinction is made between glucose and NAG except that the glucose inhibition only appears at a much higher concentration and one which is certainly not within the physiological range. Thus, the relatively low affinity of lactose synthetase for its true substrate is used to advantage to provide a mechanism for insuring the required specificity.

It is not clear from our data whether the inhibitory site is structurally removed from the catalytically active site. The fact that the relative affinities of the two sugars for catalysis are similar to their relative affinities for inhibition would be consistent with the presence of only a single site. Work is in progress which we hope may resolve this question.

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