THE CORRELATION BETWEEN THE APPARENT MOLECULAR WEIGHT AND THE ENZYMIC ACTIVITY OF LACTOSE SYNTHETASE

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A galactosyl transferase present in milk catalyses the reaction [1]:

$$\text{UDP-galactose} + N\text{-acetylglucosamine} \xrightarrow{Mn^{2+}} N\text{-acetyllactosamine} + \text{UDP}$$

The transferase is referred to as protein A, and the catalysis as transferase activity, or A activity. In the presence of a second protein, B, the transferase catalyses the reaction [2]:

$$\text{UDP-galactose} + \text{glucose} \xrightarrow{Mn^{2+}} \text{lactose} + \text{UDP}.$$

This catalysis is referred to as lactose synthetase activity, or AB activity. Protein B is α-lactalbumin [3] present in large amounts in milk [4].

Methods for isolating the galactosyl transferase rely on retention of the enzyme, in the presence of *N*-acetylglucosamine [5] or glucose [6], by α-lactalbumin which is covalently-bound to an inert matrix. The procedure is indicative of an association between the two proteins. In the initial stages of purification of the galactosyl transferase, it is separated from excess α-lactalbumin by gel-filtration. Repeated gel-filtration gave various elution volumes for the enzyme, presumably depending on the amount of α-lactalbumin still complexed with the transferase [7]. It should be possible to assess the relative amounts of α-lactalbumin present in the eluate by measuring the ability to catalyse each of the two reactions.

Work in this laboratory [8] has shown that a complex between the galactosyl transferase and α-lactalbumin can be isolated and characterised as containing the proteins in a molar ratio of 1:1. The present communication provides simple evidence for the protein-protein interaction, and shows a correlation between gel-filtration data and the type of enzyme activity.

Results and discussion

The enzyme was partially purified from bovine milk. The initial steps involved removal of cream together with the predipitate formed at pH 4.6. After adjustment to pH 7.4. with Tris, addition of $MnCl_2$ (to 40 mM) the fraction between 35 and 60 per cent saturation of ammonium sulphate contained the enzyme activity. A similar procedure was used for samples of human milk. The protein dissolved in Tris–citrate, pH 6.8, $I = 0.03$, was applied to a column of Sephadex G-100 (150 × 1 cm). The column was calibrated with blue dextran and protein markers: γ-globulin, serum albumin, ovalbumin and ribonuclease. The calibration was based on the logarithm of the molecular weight against the ratio of elution volume to void volume [9].

The enzyme assay was based on the method of Brodbeck and Ebner [2]. This is a coupled assay, relying on nucleoside diphosphokinase (present in the pyruvate kinase, Sigma Type II), pyruvate kinase and lactate dehydrogenase. It was performed in 1 ml cuvettes with 0.1 mM ATP; 1 mM PEP; 0.15 mM NADH; 12.5 mM $MnCl_2$; 50 mM Tris–HCl, pH 7.5; 50 μg/ml pyruvate kinase and 1.7 μg/ml lactate dehydrogenase. The substrates were 0.084 mM UDP-galactose and 20 mM in either *N*-acetylglucosamine (for transferase activity) or glucose (for synthetase activity). The assay

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was at a temperature of 25°C, and the oxidation of NADH was followed at 340 nm in a Hilger Uvispek.

The successive associations possible between protein B and protein A are assumed to give the species AB, AB_2, AB_3, AB_4 ...

The total molar concentration, P, is the sum of the molar concentrations of the species present:

$$P = [A] + [AB] + [AB_2] + \ldots$$

The weight concentration c, in g/l, is given by:

$$c = \bar{M}P = M_A\,[A] + (M_A + M_B)\,[AB] + (M_A + 2M_B)\,[AB_2] + \ldots$$
$$= M_A\,([A] + [AB] + [AB_2] + \ldots) + M_B\,([AB] + 2[AB_2] + \ldots)$$

$\bar{M}$ is the number-average molecular weight. For a maximum of n binding sites on A, the fraction of sites occupied Y, by B, is given by:

$$Y = \frac{[AB] + 2[AB_2] + \ldots}{n([A] + [AB] + [AB_2] + \ldots)} =$$

$$= \frac{[AB] + 2[AB_2] + \ldots}{nP}.$$

Therefore, $\bar{M} = M_A + M_B \cdot n \cdot Y$.

This equation is valid for the general case of an interaction between proteins, or between a protein and a ligand. If the molecular weight is related to the fractional saturation, the number of sites can be determined.

For lactose synthetase the fractional saturation may be related to the type of catalytic activity using certain assumptions on the basis of previous data. In the presence of α-lactalbumin the change from transferase to synthetase activity is not absolute. With the present substrate concentration (20 mM *N*-acetylglucosamine), the presence of α-lactalbumin does not alter the transferase activity [10]. Therefore, at this substrate concentration, the transferase activity is related to the total sites present, both free or complexed with B. At the concentration of glucose (20 mM) used, the synthetase activity of the A protein is zero [10]. Therefore, at this substrate concentration, the synthetase activity is related to the sites which are complexed with B.

A further requirement to obtain the fractional saturation is the relation between the two types of activity at the particular assay conditions used. Considering a notional correlation factor, r, which relates the apparent synthetase activity per complexed site to the transferase activity per site, then:

$$Y = \frac{[AB] + 2[AB_2] + \ldots\ldots}{n([A] + [AB] + [AB_2] + \ldots)} = \frac{\Sigma\,(AB)\ \text{sites}}{\Sigma\,(A + AB)\ \text{sites}}$$

$$= \frac{\text{AB activity}}{(\text{AB activity/site})} \cdot \frac{(\text{A activity/site})}{\text{A activity}}$$

$$= \frac{1}{r} \cdot \frac{\text{AB activity}}{\text{A activity}}.$$

Substitution in the expression for molecular weight:

$$\bar{M} = M_A + M_B \cdot \frac{n}{r} \cdot \frac{\text{AB activity}}{\text{A activity}}.$$

Unless excess α-lactalbumin is present the full potential synthetase activity per site is not expressed owing to dissociation of the complex. Furthermore at low α-lactalbumin concentrations and 20 mM in each monosaccharide substrate, the synthetase activity is significantly less than the transferase activity [10]. Therefore, in the present assays, the correlation factor between these activities should be less than unity.

The activities of the peak fractions are given in table 1, and the apparent molecular weight from gel-filtration is plotted against the ratio of synthetase activity to transferase activity in fig. 1. The line was calculated by a least mean squares procedure. The intercept corresponding to M_A is 47 100 ± 900 and the slope is 26 000 ± 2600. From a value of 14 200 for M_B from the composition of α-lactalbumin [11], the value of $\frac{n}{r}$ is 1.8 ± 0.2. The restrictions that n should be an integer and r significantly less than unity lead to a unique solution of $n = 1$ and $r = 0.56$.

These results are consistent with the previous inference [8] of a 1:1 molar complex formed between the A and B proteins. Although the experimental procedure is approximate the molecular weights of 47 000

Table 1
Synthetase and transferase activities after gel-filtration

Enzyme source	A activity (units/ml)	AB activity (units/ml)	AB activity / A activity	Estimated molecular weight
Human milk	60	0	0.000	47 800
Human milk	38	19	0.500	61 600
Bovine milk	21	0	0.000	42 200
Bovine milk	11	2	0.182	50 100
Bovine milk	25	6	0.240	54 300
Bovine milk	48	10	0.208	55 000
Bovine milk	24	9	0.375	55 600
Bovine milk	30	13	0.433	56 200
Bovine milk	26	11	0.423	57 500
Bovine milk	44	27	0.614	63 100

The units/ml are for the peak fraction after gel-filtration. Units correspond to nmoles of UDP produced per minute at 25°C [2]. The molecular weight was estimated from the ratio of elution volume to void volume, compared against marker proteins.

for A and hence 61 000 for the AB complex are close to previous determinations of 45 000 and 60 000 respectively [8].

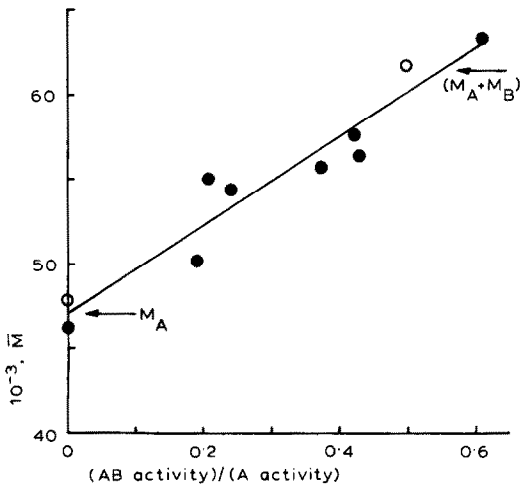

Fig. 1. Graph of apparent molecular weight, $\bar{M}$, from gel-filtration data, against the ratio of synthetase to transferase activity for the peak fraction after gel-filtration. The open circles are for enzyme from human milk and the closed circles for enzyme from bovine milk. The line was calculated by a least mean squares procedure. M_A and $(M_A + M_B)$ indicate the molecular weights for the A protein and for the AB complex respectively.

The data presented support a direct connection between the amount of B complexed with the A protein and the corresponding increase in synthetase activity.

The correlation between the synthetase activity and transferase activity depends on the assay conditions used. An increased synthetase activity would be expected at higher glucose concentrations in the reaction, but would lead to the complication that the A protein alone shows synthetase activity on increase of this substrate [10]. In the present work, the saturation function, Y, has been interpreted by reference to the comparison of synthetase and transferase activity by Klee and Klee [10].

The maximal values obtained for the apparent molecular weight and the ratio of the enzyme activities in fig. 1 were obtained on addition of α-lactalbumin to the transferase preparation, with *N*-acetylglucosamine in the buffer. They correspond to the values at saturation of the binding sites and agree with the inferences made from the slope of the line for the nature of the complex and the correlation between the activities.

The connection between molecular weight and the saturation function is of general validity, and on determination of these parameters, the method can be used for studying problems of protein–protein interaction.

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