The subcellular distribution of the A and B proteins of lactose synthetase in bovine and rat mammary tissue

Urs Brodbeck and K. E. Ebner

From the Department of Biochemistry, Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74074

SUMMARY

The subcellular distribution of the A and B proteins of lactose synthetase (EC 2.4.1.1) has been determined in bovine and rat mammary tissue. The A protein is mainly associated with the microsomal fraction, whereas the B protein is distributed between the microsomal and soluble fraction. The B protein is more readily dissociated from the microsomes than the A protein. Microsomes present in milk have the same specific activity of the A and B protein as microsomes isolated from mammary tissue.

The biosynthesis of lactose from D-glucose involves several enzymatic reactions, the last of which is catalyzed by the enzyme UDP-D-galactose:D-glucose-1-galactosyltransferase (EC 2.4.1.1). This enzyme is commonly referred to as lactose synthetase and catalyzes the following reaction.

UDP-D-galactose + α-D-glucose → lactose + UDP

Lactose synthetase was shown to exist as a microsomal enzyme in mammary glands of lactating cows or guinea pigs (1) and as a soluble enzyme in bovine milk (2, 3). Recently, the soluble enzyme from milk was demonstrated to require the presence of two proteins, called A and B, for activity and that individually these proteins did not exhibit any catalytic activity (4). The microsomal lactose synthetase could be solubilized by sonic oscillation; however, fraction B of the milk enzyme was solubilized to a greater extent than the A protein, and when microsomes were incubated under a variety of conditions, the B protein was solubilized to a greater extent than the A protein; and (c) the elution pattern on a BioGel P-30 of the A protein solubilized from microsomes and the B protein obtained from the 105,000 × g supernatant solution of a bovine mammary tissue extract corresponded to the elution pattern of the A and B proteins isolated from the soluble milk enzyme.

EXPERIMENTAL PROCEDURE

Chemicals—UDP-α-D-Gal-1-14C was synthesized enzymatically by the procedure described by Anderson et al. (5), and purity was verified by ascending paper chromatography (6) and by radioautography on "no screen" film (Eastman). The product was further checked by enzymatic conversion to UDPGlucose by UDPGal-4-epimerase (EC 5.1.3.2) and by UDP-glucuronate (EC 1.1.1.22) and appeared to be homogenous by the above criteria. More recently, UDPGal 14C (Gal-14C (U. L.)) (120 mC per mmole) was purchased from New England Nuclear. Nonradioactive UDPGald was synthesized according to the method of Moffatt and Khorana (7), Roseman et al. (8), and Moffatt (9). Other chemicals were obtained from the following sources: UTP, ATP, and pyruvate kinase (type 1, crude) from Sigma; BioGel P-30 and BioRad AG 11A8 from Bio-Rad; and Biochrome from Lövdalens Atiebolag, Stockholm, Sweden. All other chemicals were reagent grade. Mammary tissue from lactating cows was obtained from the Wilson Packing Plant, Oklahoma City, and in some cases from Dr. B. L. Larson, University of Illinois.

Methods—Protein was determined by the method of Lowry et al. (10) with bovine serum albumin as standard (Mann, Type V). Unless otherwise stated, all procedures were carried out between 0° and 4°. Mammary tissue from freshly slaughtered cows or rats was freed from excessive fat and connective tissue, quickly frozen in Dry Ice and stored at −15°.

Assay for Lactose Synthetase Activity—Enzymatic activity (millimicromoles per min) was determined either by measuring the incorporation of UDP-α-D-Gal-14C into lactose-14C or by determining spectrophotometrically the amount of UDP formed (4). Kinetic experiments are consistent with the view that the A and B proteins interact to form an enzymatically active AB complex, since A may be saturated by B and vice versa. Thus, A or B activity may be estimated in the presence of saturating amounts of the counterpart. Such assays gave a linear response.

* This research was supported in part by United States Public Health Service Grant AM-06889.

Received for publication, June 15, 1966
with respect to the concentration of the protein assayed. This type of assay is similar to the one used for estimating the enzyme subunits of tryptophan synthetase (11).

The proportionality between the rate of reaction and the amount of the limiting protein fraction was determined with the A and B proteins of the soluble lactose synthetase isolated from bovine milk as previously reported (4). In this more purified system, lactose synthetase activity was determined spectrophotometrically by assaying the amount of UDP formed according to the method of Davidson (12). Because lactose and UDP are formed in stoichiometric amounts, the activity of lactose synthetase may be expressed as the millimicromoles of lactose or UDP formed. This correspondence was obtained when either the spectrophotometric or the incorporation assay was used. The proportionality between the initial rate of reaction and the amount of the limiting protein is shown in Fig. 1 for the A protein and in Fig. 2 for the B protein.

Assays involving crude extracts of mammary tissues or bovine milk were done by the incorporation method since this assay was more sensitive and a NADH oxidase present in crude systems reduced the utility of the spectrophotometric assay. A typical incorporation mixture contained the following in a final volume of 0.1 ml: 5 μmoles of Tris-HCl at pH 7.4, 4 μmoles of MnCl₂, 2 μmoles of n-glucose, 0.5 μmole of UTP, 7.0 × 10⁻⁴ μmole of UDP-α-Gal-¹⁴C (2880 cpm), and varying amounts of enzyme. In the assays for the A protein, 0.09 to 0.23 mg of purified bovine B protein was added, whereas in the assay for the B protein, 0.14 mg of the A protein (eluate of the BioGel P-30 column) was added to the assay mixture. After 30 to 60 min of incubation at 37°, the reaction mixture was treated as previously described (4). All assays for the A or B proteins were carried out under conditions where the counterpart protein was in excess with respect to the protein under determination. The reaction was always started by the addition of UDP-α-galactose-¹⁴C. An incubation blank (no glucose) and zero time blank were necessary controls in crude systems to correct for hydrolysis of UDP-α-Gal-¹⁴C. As shown by Watkins and Hassid (1), the addition of UTP to the particulate fractions in the lactose synthetase assay was necessary to protect against excessive breakdown of UDP-α-Gal by phosphatase present. ATP may substitute for UTP in such crude systems.

Preparation of Active Particulate Fraction from Bovine Mammary Tissue—Watkins and Hassid (1) reported that lactose synthetase is associated with a particulate fraction which contains mitochondria and microsomes. A preliminary subcellular distribution study showed that the majority of the enzymatic activity was associated with the microsomal fraction. As a result of these experiments, the procedure of Watkins and Hassid was modified so that larger amounts of particles could be prepared.

Frozen mammary tissue was thawed, cut into small pieces, and passed three times through a mechanical meat grinder. One hundred-gram portions were homogenized at 0° for 20 sec in 250 ml of 0.25 M sucrose with a Virtis overhead homogenizer at a Variac setting of 80. The mixture was centrifuged at 15,000 × g for 20 min, and the supernatant solution, containing large amounts of fat, was filtered through glass wool. To increase

1 Details of the purification procedure will be presented in a subsequent publication. Recent experiments have shown that the B protein of lactose synthetase is identical with α-lactalbumin (28).

The yield of microsomes, the precipitate was resuspended in 0.25 M sucrose (approximately one-fourth of the original amount used), homogenized, and recentrifuged at 50,000 × g for 90 min; the microsomal pellet was washed twice in 0.25 M sucrose and resuspended to give a concentration of approximately 40 mg of protein per ml in 0.25 M sucrose. The amount of microsomal protein obtained varied between 70 and 340 mg/100 g of mammary tissue depending upon the quality and age of the tissue. A lower yield of microsomes was obtained from tissue that had been stored frozen for a long time. The microsomes could be stored in 0.25 M sucrose at 4° for 14 days without significant loss of enzymatic activity.

RESULTS

Solubilization of Microsomal Lactose Synthetase—Our initial attempts to solubilize the microsomal lactose synthetase from bovine mammary tissue were unsuccessful as determined by the incorporation assay (4). These methods included: extraction of acetone and butanol powders of whole tissue or microsomes (13);
Subcellular Distribution of Lactose Synthetase

Comparison of methods for solubilization of microsomal lactose synthetase

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experimental method</th>
<th>Extracting media</th>
<th>Temperature °C</th>
<th>Time min</th>
<th>Units per ml</th>
<th>Total units</th>
<th>Specific activity</th>
<th>Microsomal protein used mg</th>
<th>Efficiency of solubilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanol extraction of tissue</td>
<td></td>
<td>20 mM Tris-HCl, 20 mM MgCl₂ (pH 8.1), 20% butanol</td>
<td>25</td>
<td>45</td>
<td>8.84</td>
<td>18.5</td>
<td>4.42</td>
<td>103</td>
<td>0.18</td>
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<tr>
<td></td>
<td></td>
<td>20 mM Tris-HCl, 20 mM MgCl₂ (pH 8.1), 20% butanol</td>
<td>0</td>
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<td>3.92</td>
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<td>0.16</td>
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<td>Acetone powder extraction</td>
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<td>0.25 M Tris-HCl, 20 mM MgCl₂ (pH 7.4)</td>
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<td>60</td>
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<td>60</td>
<td>4.4</td>
<td>3.4</td>
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<td>76</td>
<td>0.46</td>
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<td>180</td>
<td>7.0</td>
<td>45.5</td>
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<td>94.5</td>
<td>0.46</td>
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<td>Extraction of microsomes with detergents</td>
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<td>0.2% Tween 80</td>
<td>0</td>
<td>60</td>
<td>1.68</td>
<td>2.8</td>
<td>0.56</td>
<td>7.3</td>
<td>0.38</td>
</tr>
<tr>
<td>Sonic oscillation of microsomes</td>
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<td>0.25 M Tris-HCl, 20 mM MgCl₂ (pH 7.4)</td>
<td>0</td>
<td>5</td>
<td>15.7</td>
<td>12.5</td>
<td>6.68</td>
<td>12.8</td>
<td>1.08</td>
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<tr>
<td></td>
<td>0.25 M Tris-HCl, 20 mM MgCl₂ (pH 7.4)</td>
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<td>17.0</td>
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<td>0.25 M Tris-HCl, 20 mM MgCl₂ (pH 7.4)</td>
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<td>27.6</td>
<td>22.2</td>
<td>17.2</td>
<td>1.90</td>
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<td>0.25 M Tris-HCl, 20 mM MgCl₂ (pH 7.4)</td>
<td>10</td>
<td>20.8</td>
<td>16.7</td>
<td>6.12</td>
<td>1.16</td>
<td>1.87</td>
<td>1.47</td>
</tr>
</tbody>
</table>

mentioned above lactose synthetase activity in intact microsomes was stimulated 2-fold by the addition of the B protein obtained from skim milk, and the solubilized lactose synthetase was greatly stimulated (10-fold or more) by the addition of the B protein. Also, evidence was obtained which showed that the soluble portion of rat or bovine mammary tissue stimulated the activity of microsomal lactose synthetase. These data suggested that there may be an unequal distribution of the A and B proteins within mammary cells, and, accordingly, the subcellular distribution of the A and B proteins of the enzyme was investigated following a modified method of Schneiter and Hogeboom (16).

Frozen mammary tissue from lactating cows or rats was cut into 2- to 5 mm dice and washed three times in 0.25 M sucrose at 4 °C. After each wash, the tissue was gently blotted between several layers of filter paper to remove the majority of the residual milk present in the tissue. Removal of residual milk is essential since the enzyme is present in milk and would appear in the 105,000 × g supernatant fraction thus giving a false distribution pattern.

After washing the slices, the bovine mammary tissue was passed through a mechanical meat grinder three times. This material (10 g) was homogenized for 30 sec with 50 ml of 0.25 M sucrose with the Virtis overhead homogenizer (Virtis setting at 80). The resulting suspension was centrifuged at 700 × g for 10 min. The precipitate was washed with 30 ml of 0.25 M sucrose and recentrifuged at 700 × g. The precipitate obtained (nuclear fraction) was suspended in 0.25 M sucrose and freed from large pieces of connective tissue by filtering the suspension through four layers of cheesecloth. The combined 700 × g supernatant solutions were centrifuged for 10 min at 5,000 × g. The precipitate, after washing in 0.25 M sucrose, yielded the mitochondrial fraction. The 5,000 × g supernatant solution was centrifuged for 60 min at 105,000 × g. The microsomal pellet was suspended in 0.25 M sucrose and the microsomal suspension and the soluble fraction were recentrifuged for 120
earlier observation that the B protein is limiting in microsomes. This distribution agrees with the
soluble fractions of the cell. In both species, the A protein is associated primarily with the microsomal frac-
tion, whereas the B protein is found in the microsomal and the soluble fractions of the cell. This distribution agrees with
the earlier observation that the B protein is limiting in microsomes.

**Gel Filtration Studies**—It was of interest to compare the elution pattern on BioGel P-30 of the A protein isolated from microsomes
and the B protein isolated from the soluble fraction with that of the A and B proteins isolated from bovine skim milk. A bovine
microsomal suspension (100 ml containing 8.4 mg of protein per ml of 0.25 M sucrose) was oscillated in a Ratheon sonic oscillator
for 20 min at 0° and then centrifuged for 90 min at 105,000 × g. The microsomal pellet was suspended in 20 mM Tris-HCl, 5 mM
MgCl₂ at pH 7.4. Fig. 3 shows the protein elution pattern ($A_{280}$) and lactose synthetase activity of the B protein when
assayed in the presence of saturating amounts of A protein obtained from bovine skim milk. The fractionation and gel filtration are described in the

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total protein</th>
<th>A protein</th>
<th>B protein</th>
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<tr>
<td>Nuclear</td>
<td>1.5</td>
<td>14.6</td>
<td>2.7</td>
<td>9.4</td>
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<tr>
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<td>15.6</td>
<td>2.8</td>
<td>9.8</td>
</tr>
<tr>
<td>Microsomal</td>
<td>4.5</td>
<td>93.5</td>
<td>21.2</td>
<td>73.8</td>
</tr>
<tr>
<td>Soluble</td>
<td>25.0</td>
<td>130.0</td>
<td>2.0</td>
<td>7.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A protein</th>
<th>B protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total units</td>
<td>Specific activity</td>
</tr>
<tr>
<td>ml</td>
<td>µmoles/min%</td>
</tr>
</tbody>
</table>

| Nuclear         | 1.5              | 14.6            | 2.7             | 9.4             |
| Mitochondrial   | 2.0              | 15.6            | 2.8             | 9.8             |
| Microsomal      | 4.5              | 93.5            | 21.2            | 73.8            |
| Soluble         | 25.0             | 130.0           | 2.0             | 7.1             |

In a similar manner, the soluble B protein was demonstrated to be associated with a low molecular weight fraction from the
P-30 column. The soluble fraction of a bovine mammary extract was made 20 mM Tris-HCl, 30 mM MnCl₂ at pH 7.4, and
the precipitate was discarded. Solid ammonium sulfate (390 g per liter) was added to obtain 60% saturation in ammonium
sulfate and the precipitate was dissolved. The supernatant solution was brought to 75% saturation in ammonium
sulfate (106 g per liter), and the precipitate was dissolved in 0.25 M Tris-HCl, pH 7.4. Eight milliliters of this solution (23 mg of
protein per ml) were passed through the BioGel P-30 column (3 × 160 cm) equilibrated and eluted with 20 mM Tris-HCl, 5 mM
MgCl₂, pH 7.4. Fig. 4 shows the protein elution pattern ($A_{280}$) and lactose synthetase activity of the B protein when
assayed in the presence of saturating amounts of A protein obtained from bovine skim milk. The elution pattern of the B
protein from the soluble fraction with that of the A and B proteins isolated from bovine skim milk. The elution pattern of the B
protein corresponds to the elution pattern of B protein obtained from
bovine skim milk. The A protein eluted with the majority of the high molecular proteins, and its elution volume corresponded to
the A protein isolated from soluble lactose synthetase of skim milk.

In a similar manner, the soluble B protein was demonstrated to be associated with a low molecular weight fraction from the
P-30 column. The soluble fraction of a bovine mammary extract was made 20 mM Tris-HCl, 30 mM MnCl₂ at pH 7.4, and
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protein per ml) were passed through the BioGel P-30 column (3 × 160 cm) equilibrated and eluted with 20 mM Tris-HCl, 5 mM
MgCl₂, pH 7.4. Fig. 4 shows the protein elution pattern ($A_{280}$) and lactose synthetase activity of the B protein when
assayed in the presence of saturating amounts of A protein obtained from bovine skim milk. The elution pattern of the B
protein from the soluble fraction of bovine mammary tissue corresponds to the elution pattern of B protein obtained from
the soluble milk enzyme.

Fig. 3. BioGel P-30 filtration of the A protein of solubilized lactose synthetase from bovine mammary microsomes. The solu-
bilization, fractionation, and gel filtration are described in the

text. Fractions, 5.0 ml each, were collected after the first 210 ml
passed through the column. ○, protein distribution in elute fractions ($A_{280}$); ●, lactose synthetase activity of the A protein
in the presence of 17.5 units of the B protein (22.5 µg of B). The incorporation assays were carried out as described in “Methods.”

Fig. 4. BioGel P-30 filtration of the B protein of lactose synthetase from bovine mammary tissue. The fractionation and gel filtration are described in the text. Fractions, 4.3 ml each, were collected after the first 300 ml passed through the column. ○, protein distribution in elute fractions ($A_{280}$); ●, lactose synthetase activity of the B protein in presence of 2.37 units of the A protein (15.0 µg of A). The incorporation assays were carried out as described in “Methods.”
was removed by centrifugation and discarded. An ultraviolet spectrum of this partially purified material resembled the spectrum obtained from the purified B protein from skim milk. No further attempts were made to characterize the B protein from the soluble mammary tissue extract since it was isolated in only small amounts from this tissue.

**Dissociation of Microsomal Lactose Synthetase**—As a result of the subcellular distribution study, it was apparent that the A and B proteins necessary for lactose synthetase activity were both present in microsomes. However, the majority of the B protein was found in the soluble portion of the cell, and the B protein seemed to be associated weakly with the microsomal protein. If this were true, then it should be possible to find conditions whereby the microsomal B protein is more specifically released into the soluble fraction than is the A protein. Attempts were made to dissociate the A and B proteins of microsomal lactose synthetase under a variety of mild conditions.

Bovine mammary microsomes were suspended in 0.25 M sucrose or in 20 mM Tris-HCl at pH 7.4 and incubated for 1 hour at 0° in the presence of EDTA, acetone, KCl, and ammonium sulfate. The incubation mixture was then centrifuged for 90 min at 105,000 x g; the remaining microsomal pellet was washed with 0.25 M sucrose or 20 mM Tris-HCl, pH 7.4, and finally dispersed in the same solution. The combined 105,000 x g supernatant solutions were dialyzed overnight against 500 ml of 0.25 M sucrose or in 20 mM Tris-HCl, pH 7.4 (changed three times), and then concentrated to approximately 2 ml with Biodryex.

Both the microsomal and soluble fractions were assayed for the A and B protein of lactose synthetase. Incubation blanks containing no glucose showed that in the solubilized fractions, even in the absence of UTP, only small amounts of neutral sugars were formed from endogenous pyrophosphatase activity and in these assays UTP could be omitted. Table III shows the effect of EDTA, acetone, KCl, and ammonium sulfate on the release of the A and B proteins of microsomal lactose synthetase when microsomes were incubated in either 0.25 M sucrose or 20 mM Tris-HCl, pH 7.4. Comparison of the percentage distribution of the A and B proteins between the remaining microsomes and the solubilized material revealed that under identical experimental conditions the B protein was released into the soluble fraction to a greater extent than the A protein. When incubated in 0.25 M sucrose, essentially no A activity was found in the soluble fraction even in the presence of EDTA, acetone, KCl, or combinations thereof, whereas up to 62% of the total microsomal A activity was solubilized when the microsomes were incubated in presence of 20 mM Tris-HCl, pH 7.4, suggesting that under conditions of low osmotic pressure, microsomal protein may be partially solubilized. The addition of EDTA, acetone, and ammonium sulfate resulted in only a slight increase in solubilized A activity whereas up to 95% of the total microsomal B activity was released under identical conditions. These findings are consistent with all earlier observations that the B protein is limiting in microsomes.

**Lactose Synthetase in Microsomes Isolated from Bovine Milk**—As previously indicated, the A and B proteins of lactose synthetase exist in a soluble form in bovine skim milk, whereas in mammary tissue, lactose synthetase activity is associated mainly with the microsomal fraction. Only small amounts of lactose synthetase activity were found in the soluble fraction of bovine or rat mammary tissue.

Milk contains microsomes which are associated mainly with the cream fraction, and these microsomes were examined for their lactose synthetase activity as was the distribution of the A and B proteins in whole milk.

Bovine milk microsomes were isolated according to a procedure described by Morton (17). Fresh whole milk (100 ml) was cooled

<table>
<thead>
<tr>
<th>Incubation mixtures</th>
<th>A protein</th>
<th>B protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microsomes</td>
<td>Soluble fraction</td>
</tr>
<tr>
<td></td>
<td>Total units</td>
<td>Specific activity</td>
</tr>
<tr>
<td>0.25 M sucrose</td>
<td>103 1.5</td>
<td>103 1.5</td>
</tr>
<tr>
<td>0.25 M sucrose, 10 mM EDTA</td>
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<td>123 3.5</td>
</tr>
<tr>
<td>0.25 M sucrose, 20% acetone</td>
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<td>0.25 M sucrose, 10 mM EDTA</td>
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<td>0.25 M sucrose, 20 mM KCl</td>
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<td>125 2.3</td>
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<td>0.25 M sucrose, 10 mM EDTA</td>
<td>125 2.3</td>
<td>125 2.3</td>
</tr>
<tr>
<td>20 mM Tris-HCl (pH 7.4)</td>
<td>3.5 1.1</td>
<td>3.5 1.1</td>
</tr>
<tr>
<td>20 mM Tris-HCl (pH 7.4), 10 mM EDTA-Tris (pH 7.4)</td>
<td>3.5 1.1</td>
<td>3.5 1.1</td>
</tr>
<tr>
<td>20 mM Tris-HCl (pH 7.4), 10% acetone, 20% acetone, 0.2 M ammonium sulfate</td>
<td>3.5 1.1</td>
<td>3.5 1.1</td>
</tr>
</tbody>
</table>
to 4° and centrifuged at 2,500 × g for 30 min. The cream was carefully removed with a spatula and washed by suspending it in 50 ml of H2O. After centrifugation for 20 min at 2,500 × g, the cream was again suspended in 50 ml of H2O and churned by shaking for 20 min at room temperature. The buttermilk was decanted off and centrifuged together with the washings at 78,000 × g for 90 min. The microsomal precipitate was washed in 20 mM Tris-HCl, 5 mM MgCl2 at pH 7.4, recenterfuged at 78,000 × g for 90 min, and suspended in the same buffer. The skin milk was centrifuged at 16,000 × g for 30 min and the precipitate (heavy casein) was discarded. The supernatant solution was centrifuged two more times at 78,000 × g and the precipitate (light casein) was discarded. Table IV shows the distribution of the A and B protein of lactose synthetase in bovine whole milk. Although the soluble fraction contains approximately 90% of the activity of the two proteins of lactose synthetase, the microsomes isolated from cream had the ability to incorporate the galactose moiety of UDP-α-C-Gal into 14C-lactose. The distribution observed in mammary tissue was similar to the A and B proteins in the milk microsomal particles were similar to those of the particulate mammary enzyme. Because of coprecipitation, some lactose synthetase activity was found in the light and heavy casein fractions. However, after washing these precipitates, the activity was found in the soluble fraction.

**DISCUSSION**

Recently, several enzymes have been demonstrated to require two protein fractions for maximum enzymatic activity (18, 19, 20, 21). In all of these cases, the enzymes were isolated from microbial sources. Lactose synthetase appears to be the first mammalian enzyme shown to require two protein fractions for activity (4). Furthermore, this enzyme is unique in that the A and B proteins of milk microsomes are both soluble, whereas in mammary tissue the A protein is principally associated with the microsomes and the B protein is distributed between the microsomes and the soluble portion of the cell. The distribution observed in mammary tissue may be misleading since the B protein may have been dissociated from the AB complex in microsomes during the isolation procedure since studies on the dissociation of microsomal B showed that it was bound weakly to microsomes. The A protein was demonstrated by gel filtration to be present in solubilized microsomes from bovine mammary tissue, and the B protein was shown to be present in a partially purified soluble extract of bovine mammary tissue. It would appear that the A and B proteins in whole tissue were similar to the A and B proteins present in milk. Milk also contains microsomal particles and about 1% of the total lactose synthetase activity of milk was present in these particles. The specific activities of the A and B proteins in the milk microsomal particles were similar to the tissue microsomes, indicating that the milk microsomes were similar to tissue microsomes, an observation previously made by Morton (17) with other milk microsomal enzymes. Morton found that the lipoprotein particles from milk had chemical and enzymatic properties similar to those of cytoplasmic microsomes and called them "milk microsomes." He concluded that the microsomes of milk are derived directly from the secretory cells of the mammary gland. On the basis of these observations and the results presented here, it appears that lactose synthetase from mammary tissue is identical with the enzyme from milk. The exact manner by which lactose synthetase becomes solubilized in milk is not clear at the present time although it would appear that it arises from the microsomal enzyme.

In order to get an understanding of the forces holding the subunits of lactose synthetase together, the microsomal enzyme was subjected to a variety of mild chemical treatments. Incubation in the presence of 10 mM EDTA (disodium) released 31% of the B protein into the soluble fraction, whereas 20 mM KCl (having the same ionic strength as 10 mM EDTA (disodium)) only released 14% (Table III). These results suggested that, to some extent, the B protein may be bound in the microsomes to the A protein by Mg++ or Mn++ since these two cations were shown to activate lactose synthetase (2). The importance of metallic ions in binding together protein subunits has long been recognized. Kagi and Vallee (22) reported that α-phenanthroline will cause the dissociation of yeast alcohol dehydrogenase (EC 1.1.1.1) by forming a chelate complex with Zn++. Amylase from Bacillus subtilis dissociated upon removal of Zn++ and dimerization ensued when the Zn++ was replaced (23). Ca++ has been implicated in the association of the four different casein fractions (24), and there is evidence that trypsin dissociates in the absence of Ca++. (25).

In many enzymes having a mono- or diphosphate ester as substrate, the divalent cations also are required for the binding of the substrate to the enzyme. Thus in lactose synthetase, Mg++ or Mn++ may fulfill a dual role. They may function as a structural component of the active enzyme and may be required as participants in the formation of the enzyme-substrate complex. Such a dual role has been demonstrated for Mg++ in yeast enolase by Brewer and Weber (26) and in rabbit muscle enolase by Winstead and Wold (27).

Significant dissociation of the microsomal lactose synthetase also was observed in the presence of 20% acetone. In 0.25 M sucrose and 20% acetone the microsomal B protein was released to 23% into the soluble fraction. The addition of 10 mM EDTA to the acetone containing incubation mixture caused an increase in solubilization up to 62%. In 20 mM Tris-HCl, pH 7.4, the B protein was released into the soluble fraction up to 78% and 90% under similar conditions of incubation (Table III). The
addition of an organic solvent such as acetone seemed to have a marked effect on the dissociation of lactose synthetase subunits. Winstead and Wold (27) observed dissociation of rabbit muscle enolase in the presence of 10 mM EDTA and 20% aqueous dioxane or 20% aqueous acetone. Upon dialysis, the native enzyme could be reformed.

Recently, it was shown that α-lactalbumin can substitute for the B protein of lactose synthetase in the enzymatic rate assays at identical protein concentrations (28). Gordon and Ziegler (29) have determined the amino acid composition of crystalline bovine α-lactalbumin and reported 23 acidic groups in excess of the basic groups. The fact that α-lactalbumin crystallizes as a salt (30) agrees with the observation that EDTA aids in dissociation of the AB complex by chelating Mg$^{2+}$ or Mn$^{2+}$. These results lead to the conclusion that the microsomal lactose synthetase is readily dissociable under conditions in vitro at low temperatures which is in accordance with the fact that the soluble milk enzyme is easily separable into the two protein fractions, A and B.

REFERENCES

The Subcellular Distribution of the A and B Proteins of Lactose Synthetase in Bovine and Rat Mammary Tissue
Urs Brodbeck and K. E. Ebner


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