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GALACTOSYLTRANSFER IN MOUSE MASTOCYTOMA: PURIFICATION AND PROPERTIES OF *N*-ACETYLLACTOSAMINE SYNTHETASE

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SUMMARY

N-Acetyllactosamine synthetase (UDPgalactose: *N*-acetylglucosamine galactosyltransferase) was demonstrated in a microsomal fraction from mouse mastocytoma. A soluble enzyme fraction, recovered after treatment of the particulate enzyme with detergent and alkali, was further purified by affinity chromatography. The enzyme was eluted behind the main protein peak after chromatography on a column of Sepharose 4B, substituted with a derivative of *N*-acetylglucosamine. Under the conditions tested, however, only 15% of the enzyme applied to the column was recovered. Improved yields of the purified enzyme were obtained by chromatography of the solubilized enzyme on Sepharose gels containing α-lactalbumin. About one-third of the enzyme applied to such a column was adsorbed and purified approximately 570-fold.

The purified enzyme was devoid of β-galactosidase (EC 3.2.1.23) as well as the galactosyltransferases previously shown to be present in the mastocytoma tissue. Some kinetic parameters of a purified enzyme fraction have been determined.

INTRODUCTION

Recent studies with a cell-free system derived from mouse mastocytoma have shown the presence of two galactosyltransferases implicated in heparin biosynthesis[1] as well as a UDPgalactose: lipid galactosyltransferase with an as yet undefined function[2]. These reactions have been investigated with endogenous acceptors for galactose or with low-molecular weight substances which have served as exogenous substrates in model reactions for galactosyl transfer. However, partial acid hydrolysis of [^{14}C]galactose-labelled trichloroacetic acid-precipitable material showed the presence of a compound with the chromatographic properties of *N*-acetyllactosamine which constituted a major portion of the neutral radioactive oligosaccharide fragments[1]. In addition, preliminary evidence was presented for the transfer of galactose from UDPgalactose to free *N*-acetylglucosamine, yielding *N*-acetyllactosamine.

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In this communication, a more thorough study of the *N*-acetyllactosamine synthetase present in mouse mastocytoma is presented. The enzyme has been solubilized by treatment of the microsomal particles with detergent and alkali and purified approximately 570-fold. During the purification process, the enzyme was separated from the galactosyltransferases previously described in this system[1,2].

MATERIALS AND METHODS

Chemicals

UDP[^{14}C]Galactose was purchased from New England Nuclear, Frankfurt, Germany, or from The Radiochemical Centre Amersham, England. Unlabelled sugar nucleotides were obtained from Sigma Chemical Co., St. Louis, Mo., USA. 1-Ethyl-3-(3-di-methylaminopropyl)-carbodiimide was obtained from Pierce Chemical Company, Rockford, Ill. USA. 6-Amino-1-hexanol and α-lactalbumin were supplied by Koch-Light Laboratories, Colnbrook, Bucks., England. *Escherichia coli* β-galactosidase (EC 3.2.1.23) was purchased from Boehringer, Mannheim, Germany. Silica gel GF_{254} was a product of Merck AG, Darmstadt, Germany.

4-*O*-β-D-Galactosyl-D-xylose was synthesized as described[3]. *N*-Acetyllactosamine was isolated from keratan sulphate as described[4].

Analytical methods

NMR spectra were recorded with a Varian A-60 A spectrometer. Hexosamine was determined as described by Boas[5] after hydrolysis in 4 M HCl at 100 °C for 14 h. Protein was determined by the method of Lowry *et al.*[6] with human γ-globulin as standard. Digestion with β-galactosidase was carried out as described[1].

Descending paper chromatography was performed in (a) butan-1-ol–ethanol–water (10:1:2, by vol.) or (b) ethyl acetate–acetic acid–water (3:1:1, by vol.). Paper electrophoresis was carried out in Buffer (c), 0.046 M acetic acid–0.08 M pyridine, pH 5.3, at 80 V/cm for 60–90 min. Paper strips were stained with ninhydrin[7] or with a silver dip reagent[8]. Thin-layer chromatography on silica gel GF_{254} was carried out in (d) chloroform–methanol (95:5, by vol.). Spots were localized by their ultraviolet absorption characteristics or by charring with 8% H_2SO_4.

Paper chromatograms or electrophoretograms were analysed for radioactivity with a Packard model 7201 strip scanner. Radioactive products were eluted with water and quantitated with a Beckman model LS 250 liquid-scintillation spectrometer.

Vertical polyacrylamide gel electrophoresis was performed in glass tubes (0.5 cm × 7.5 cm), using 0.4 M Tris–HCl buffer, pH 8.9, with 5% acrylamide in the running gels. The current (2 mA/tube) was maintained for 2.5 h and the gels were stained with Coomassie Brilliant Blue[9].

Synthesis of N-carbobenzoxy-6-amino-1-hexanol (A)

A sample (11.7 g; 0.1 mole) of 6-amino-1-hexanol was dissolved in 100 ml of 2 M NaOH in a round bottom, three-necked vessel equipped with a mechanical stirring device. Over a period of 20 min, benzyl chloroformate (22 ml; 0.15 mole) and 4 M NaOH (63 ml) were added *via* dropping funnels. The mixture was subsequently made acid to Congo red by addition of approximately 70 ml of 6 M HCl. The reaction pro-

duct was extracted with 3 × 100 ml of ethyl ether and crystallized from chloroform at −20 °C. Recrystallization from ethanol gave 17 g (77%) A, m.p. 83–84 °C. The NMR spectrum of the product was in agreement with that expected for A. Found (%): C, 66.72; H, 8.26; O, 19.21; N, 5.62. $C_{14}H_{21}O_3N$ requires (%): C, 66.9; H, 8.42; O, 19.1; N, 5.57. The product did not stain with ninhydrin but gave a single spot which was visualized by ultraviolet irradiation after thin layer chromatography on silica gel GF_{254} (Solvent d). Catalytic reduction (see below) produced a substance with staining properties and migration rate (paper electrophoresis, Buffer c) identical with 6-amino-1-hexanol.

Synthesis of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranosyl bromide (B)

A sample (8.5 g) of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[10] was dissolved in 10 ml of glacial acetic acid and red phosphorus (1.2 g) was added. The stirred reaction mixture was cooled on ice and bromine (7.2 g) was added in a dropwise manner followed by water (1.4 g). The vessel was left at room temperature for 2 h and poured over 50 ml of ice-cold, ethanol-free chloroform. Ice-water (15 ml) was added and the chloroform layer was quickly washed with cold, saturated $NaHCO_3$ solution (2 × 30 ml) and water (2 × 30 ml). The chloroform extract was dried with anhydrous Na_2SO_4 (15 min) and filtered through a pad of Celite (B).

Synthesis of 1-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-amino-1-hexanol (C)

Compound C was synthesized by condensing (A) and (B) in a Koenigs–Knorr reaction[11] followed by removal of the protecting group. The filtrate (B) was mixed with Drierite (5 g) and iodine and immediately added to a solution of A (5 g) which had been stirred in the dark with fresh Ag_2O (15 g) and Drierite (10 g) in 30 ml of anhydrous, ethanol-free chloroform. Care was taken to initiate the condensation reaction less than 30 min after work-up of the acetobromo sugar[12].

After continued stirring in the dark for 24 h, the reaction mixture was filtered through a pad of Celite which was washed with chloroform. After treating the filtrate with 100 ml of 2% aqueous $Na_2S_2O_3$, the chloroform phase was dried over $CaCl_2$, filtered and evaporated to a syrup. On thin-layer chromatography on silica gel (Solvent d), the product migrated slightly ahead of *N*-carbobenzoxy-6-amino-1-hexanol. The syrup was dissolved in 50 ml of Solvent d and 10-ml aliquots were chromatographed on a column (5 cm × 24 cm) of silica gel, eluted with the same solvent at a rate of 60 ml per h. 18-ml fractions were collected and analysed by thin-layer chromatography. The product emerged at Fractions 17–25 which were pooled and concentrated. The syrup was suspended in 50 ml of methanol and 10 ml of 2 M NaOH were added. After 5 h at room temperature, the mixture was neutralized by Dowex 50 (H^+ form) and analysed by thin-layer chromatography (Solvent d). The mixture contained the deacetylated product as well as some *N*-carbobenzoxy-6-amino-1-hexanol. The impurities were removed by renewed chromatography on the silica gel column under the conditions described above. The deacetylated product was adsorbed to the gel and then eluted with ethyl acetate–methanol (2:1, by vol.). The pure product on NMR showed a doublet (1 H τ = 5.6 ppm, J = 7.5 Hz in $C^2H_3O^2H$) attributed to the anomeric proton and had $[\alpha]_D^{25}$ −6 ° (*c* 1.0, ethanol). The high coupling constant (J 7.5 Hz) and the low optical rotation indicate a β-pyranosidic configuration.

To remove the protecting *N*-carbobenzoxy group, the compound was dissolved

in 100 ml of 50% aqueous ethanol and reduced with 0.4 g 10% palladium–charcoal catalyst in an atmosphere of hydrogen. After the hydrogen uptake had ceased (15 ml, corresponding to approximately 0.65 mmole of product), the solution was filtered and analysed by paper electrophoresis (Buffer c). After staining with ninhydrin, a single spot with a mobility relative to 6-amino-1-hexanol of 0.70 was observed. The substance was an efficient acceptor for galactose when incubated with UDPgalactose and galactosyltransferase from mouse mastocytoma (see below). The compound was used to prepare an affinity adsorbent as described below.

Preparation of affinity adsorbents

Succinylaminoethyl-Sepharose was prepared as outlined by Cuatrecasas[13]. A solution (5 ml) containing 0.23 g of 1-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-amino-1-hexanol (Compound C described above) was adjusted to pH 5.0 and mixed with an equal volume of the substituted gel. Subsequently, a solution of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (0.5 g, dissolved in 3 ml of water) was added over a period of 5 min. The mixture was stirred for 36 h at room temperature, followed by careful washing of the gel until concentrated aliquots of the washings gave a negative colour reaction for hexosamine (see *Analytical methods)*. Aliquots of the gel were also hydrolysed and analysed for hexosamine. A yield of 0.5 μmole of coupled ligand per ml of packed gel was calculated.

α-Lactalbumin was attached directly at pH 10 to cyanogen bromide activated Sepharose gel as described[13]. The protein solution (45 mg, dissolved in 50 ml of 0.1 M Na_2CO_3 solution) was allowed to react with the activated gel (50 ml) overnight. Protein analysis of the washings showed that approximately 22 mg of α-lactalbumin had been attached to the gel.

Enzyme source

A mast cell tumor was maintained in the solid state in (A/Sn x Leaden)F_1 mice by subcutaneous or intramuscular transplantation every 10 days. The tumors (about 20 g of tissue) were removed and homogenized in a Virtis 45 homogenizer with 2 vol. of cold buffer containing Tris–acetate (12 mM), pH 7.6, KCl (50 mM) and EDTA (0.25 mM). The homogenate was subjected to centrifugation at 10 000 × *g* for 10 min and the resulting supernatant was further centrifuged at 100 000 × *g* for 60 min. The microsomal fraction which had sedimented was suspended in 12 ml of cold buffer and treated with Tween 20 and NH_4OH as described[1]. After centrifugation at 159 000 × *g* for 2 h, the top 10 ml from each tube were withdrawn and used as solubilized enzyme.

Enzyme assays

The crude enzyme was assayed by a combination of paper electrophoresis and paper chromatography as reported earlier[1]. The samples were spotted on strips (4 cm × 40 cm) of Munktell 316 paper and subjected to electrophoresis in Buffer c for 60 min. After drying the paper strips, the neutral fraction which had migrated approximately 5 cm towards the cathode on account of endosmotic flow, was subjected to chromatography in Solvent a (36 h). After staining the guide strip, the distribution of the radioactivity was determined with a strip scanner. Finally, the product was eluted and quantitated by liquid scintillation methods.

The purified enzyme, prepared as described below, was found to contain no β-galactosidase activity, and *N*-acetyllactosamine constituted all the radioactivity of the neutral fraction after electrophoresis. Therefore, a simpler assay procedure could be employed with such enzyme preparations. The incubation mixtures were diluted with water to 1 ml and passed through columns (0.5 cm × 2 cm) of Dowex 1-X2 (acetate form, 200–400 mesh). The combined effluent and washings were counted directly in the liquid scintillation spectrometer. With the purified enzyme, the two methods gave identical results.

Other galactosyltransferases occurring in the mastocytoma preparation were determined as described[1,2].

Chromatography on substituted Sepharose gels

Solubilized galactosyltransferase was extensively dialysed against the Tris–acetate buffer described above, containing $MnCl_2$ (20 mM), concentrated by pressure dialysis and incubated with UDPgalactose (0.12 mM) for 2 h at 37 °C. After removal of UDPgalactose by dialysis, the preparation (0.5 ml, 12 mg of protein) was applied to a column (1 cm × 6 cm) of *N*-acetylglucosamine-substituted Sepharose gel. The column was eluted with the Tris–acetate buffer (see above) containing 20 mM $MnCl_2$ at a rate of 6 ml per h. 1-ml fractions were collected and assayed for protein and for enzyme activity.

Chromatography on gels substituted with α-lactalbumin was similar to the procedure described above with the exception that *N*-acetylglucosamine (5 mM) and $MnCl_2$ (5 mM) were included in the eluting buffer. The column (1 cm × 7 cm) was eluted at a rate of 18 ml per h and fractions (3.4 ml) were collected. After 30 ml of buffer had passed the column, elution was continued with buffer lacking *N*-acetylglucosamine[14].

RESULTS

Transfer of galactose from UDPgalactose to N-acetylglucosamine

Fig. 1 shows product formation from *N*-acetylglucosamine and UDPgalactose when incubated with galactosyltransferase from mouse mastocytoma. With concentrated enzyme solutions, more than 50% of the labelled galactose was transferred to *N*-acetylglucosamine during 60 min. The product showed migration properties (Solvents a and b) which were indistinguishable from those of 4-*O*-β-D-galactosyl--2 acetamido-2-deoxy-D-glucose. Furthermore, the substance was cleaved by β-galactosidase, yielding galactose as the only radioactive product. Crude enzyme preparation from mastocytoma tissue also effected the hydrolysis of the isolated compound, indicating the presence of β-galactosidase activity.

On incubating unlabelled UDPgalactose and ^{14}C-labelled *N*-acetylglucosamine (0.05 μmole; 10 Ci/mole), a product was formed with chromatographic properties identical with those observed for the substance described above. This material was also degraded by β-galactosidase, leaving *N*-acetylglucosamine as the only radioactive product (paper chromatography, Solvent a).

Taken together, the data presented above suggest the presence of a UDP-galactose: *N*-acetylglucosamine galactosyltransferase in the mastocytoma prepa-

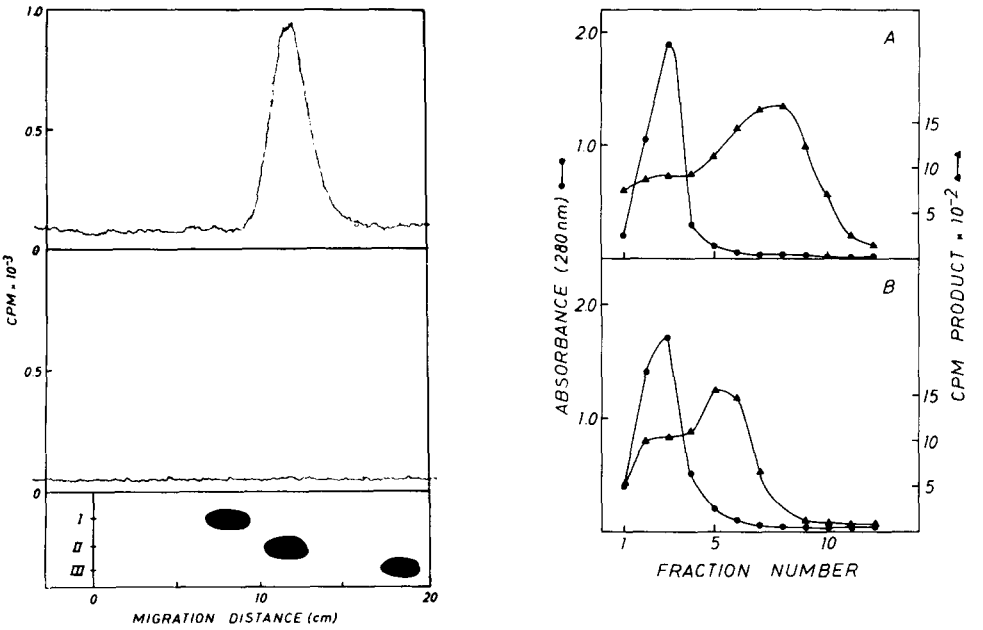


Fig. 1. (Upper) Transfer of galactose from UDPgalactose to *N*-acetylglucosamine in mouse mastocytoma. Standard incubation conditions were, UDPgalactose (0.025 μCi; 5 Ci/mole), *N*-acetylglucosamine (1 μmole), $MnCl_2$, (0.15 μmole) and purified enzyme (5.5 μg of protein), in a total volume of 0.06 ml. After 60 min at 37 °C, analysis was carried out as described in Materials and Methods. After paper electrophoresis in Buffer c, the strips were subjected to chromatography in Solvent a. (To calculate R_F-values, a correction for the endosmotic migration during paper electrophoresis should be introduced). (Lower) Control incubation with omission of *N*-acetylglucosamine in the incubation mixture. The standards indicated on the guide strip are: (I), 4-*O*-β-D-galactosyl-D-xylose; (II) 4-*O*-β-D-galactosyl-2-acetamido-2-deoxy-D-glucose; and (III) D-galactose.

Fig. 2. (A) Chromatography of solubilized *N*-acetyllactosamine synthetase on Sepharose 4B, substituted with *N*-acetylglucosamine residues, as described in Materials and Methods. Aliquots (0.05 ml) of the effluent fractions were incubated with *N*-acetylglucosamine (1 μmole) and UDP-galactose (0.025 μCi; 190 Ci/mole) for 3 h at 37 °C. (B) Chromatography of the solubilized enzyme after equilibrating the column with *N*-acetylglucosamine. Elution was carried out with buffer containing *N*-acetylglucosamine (16 mM) and aliquots were incubated with UDPgalactose (0.025 μCi; 190 Ci/mole) for 3 h and analyzed for product.

ration. The synthesis of the disaccharide, *N*-acetyllactosamine, from the requisite precursors has been reported to occur in a number of tissues[4,15–17].

Purification of N-acetyllactosamine synthetase by chromatography on N-acetylglucosamine substituted gel

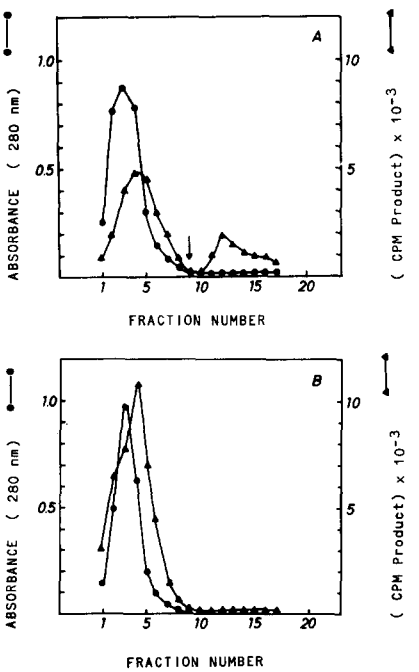
Fig. 2A shows the pattern obtained when the solubilized enzyme was chromatographed on a column of succinyl aminoethyl Sepharose gel to which 1-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-amino-1-hexanol had been covalently attached. Under the conditions tested, the enzyme was not adsorbed to the gel but appeared in a retarded position as compared to the bulk of the protein. If *N*-acetylglucosamine was included in the eluting buffer, the enzyme showed a decreased affinity for the covalently linked substrate, as may be seen from Fig. 2B. Chromatography of

solubilized enzyme which had not been preincubated as described in Materials and Methods invariably resulted in a poorer resolution.

Although a substantial purification was achieved in the single step procedure described above, recoveries were but moderate with the preincubated enzyme. The purified enzyme thus represented only about 15% of the total activity which had been applied to the column. It should be noted that only a small portion of the enzymatic activity was eluted from the column by raising the pH of the buffer (*cf.* ref. 18). Higher yields (approximately 50%) but poorer resolution were obtained when Tween 20 (0.02%) was included in the eluting buffer. However, improved recoveries were obtained by chromatography of the solubilized enzyme on columns substituted with α-lactalbumin as described below.

Purification of lactose synthetase on α-lactalbumin–Sepharose 4B gel

Fig. 3A shows the elution pattern obtained when the solubilized enzyme was chromatographed on a column of Sepharose 4B, substituted with α-lactalbumin About two-thirds of the enzyme was eluted at essentially the same position as the main protein peak, whereas one-third remained adsorbed to the column. The adsorbed enzyme fraction was eluted following removal of *N*-acetylglucosamine from the buffer



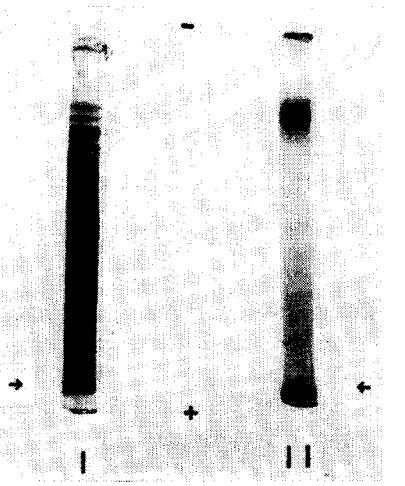


Fig. 3. (A) Chromatography of solubilized enzyme on Sepharose gel substituted with α-lactalbumin. The bulk of the protein was eluted with buffer containing *N*-acetylglucosamine (5 mM). At fraction 9 (↓) elution was started with buffer lacking this monosaccharide. (B) Pattern obtained in the absence of *N*-acetylglucosamine in the eluting buffer.

Fig. 4. Analysis by polyacrylamide electrophoresis of protein fractions equivalent to Tube 3 (I) and pooled and concentrated Tubes 11–17 (II) from the chromatogram described in Fig. 3A. The arrows indicate the migration of a dye marker.

TABLE I

PURIFICATION OF *N*-ACETYLLACTOSAMINE SYNTHETASE FROM MOUSE MASTOCYTOMA

Enzyme fraction	*Yield (%)*	*Spec. act. (cpm/mg protein × 10^{-6})*	*Purification*
Crude homogenate	100	0.017	1.0
100 000 × *g* particulate fraction	69	0.075	4.5
159 000 × *g* supernatant fraction	48	0.080	4.7
Fractions 11–17 (Fig. 3)	16	9.70	570

(*cf.* ref. 14). As is evident from Fig. 3B, no specific adsorption of the enzyme occurred in the absence of *N*-acetylglucosamine in the eluting buffer. Fractions 11–17 were pooled and concentrated by pressure dialysis and analysed for enzyme activity and for protein. Table I shows that the average specific activity for these fractions exceeded that recorded for the crude homogenate by a factor of 570.

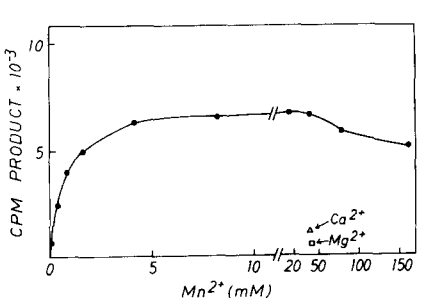
The purified enzyme contained no endogenous acceptor for galactose. Furthermore, the UDPgalactose:xylose and the UDPgalactose:Gal-4-Xyl galactosyltransferases previously described[1] were eluted with the main protein peak and were thus separated from the *N*-acetyllactosamine synthetase which had been adsorbed to the column. On polyacrylamide gel electrophoresis, the concentrated purified enzyme fraction showed a single band staining with Coomassie Blue, whereas multiple protein zones were observed with fractions eluted earlier in the chromatogram (Fig. 4).

*Kinetic properties**

Product formation from UDPgalactose and *N*-acetylglucosamine was proportional to time for 3 h but proceeded at approximately the same rate for at least 6 h. Within the range tested (0–0.25 mg protein/ml), synthesis of *N*-acetyllactosamine was proportional to the protein concentration. Maximal product formation was recorded at an acceptor concentration of about 10 mM *N*-acetylglucosamine. At higher substrate levels, a decreased synthesis of product was observed (*cf.* ref. 4). An approximate K_m value for *N*-acetylglucosamine of $1.0 \cdot 10^{-3}$ M was calculated. At fixed acceptor concentration, the K_m for UDPgalactose was estimated to $2.5 \cdot 10^{-4}$ M. Divalent cations, notably manganese, were necessary for product formation (Fig. 5). This ion could partially be replaced by Mg^{2+} or by Co^{2+}. Although active over a wide pH range with an optimum around pH 8.2 (Fig. 6), the purified enzyme was not stable to extreme pH values. Whereas treatment of the microsomal membranes in the pH range 10–11 for a short period of time did not appear to diminish the total enzyme activity, a similar exposure of the purified enzyme to pH values above 9 or below 5 followed by titration to pH 8.0, destroyed most of the enzymatic activity.

In Fig. 7, heat inactivation of the *N*-acetyllactosamine synthetase is illustrated. It is seen that presence of UDPgalactose seems to stabilize the enzyme somewhat against heat denaturation whereas the amount of product formed with enzyme which had been heat-treated in the presence of *N*-acetylglucosamine did not differ significantly from the values obtained from the control incubations.

* For determination of kinetic parameters, a 200-fold purified enzyme fraction was utilized in most experiments. No evidence was obtained, however, suggesting that this enzyme preparation possessed properties distinct from those of the 570-fold purified enzyme fraction.



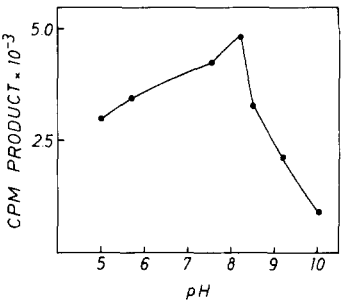
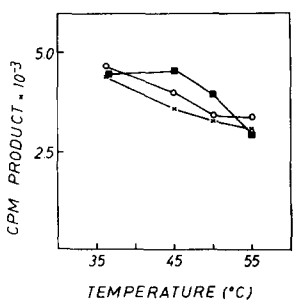


Fig. 5. Product formation as a function of Mn^{2+} concentration. Standard reaction conditions (see Fig. 2) except for divalent cations were employed.

Fig. 6. pH-profile for *N*-acetyllactosamine synthetase from mouse mastocytoma. The purified enzyme was titrated to an appropriate pH value by addition of small amounts of NH_4OH or acetic acid. Aliquots were then incubated with the requisite precursors under the conditions described in Fig. 2.

Substrate specificity

The purified enzyme showed activity only with *N*-acetylglucosamine or derivatives containing this monosaccharide as nonreducing endgroup. Recent experiments[19–22] have indicated that UDPgalactose: *N*-acetylglucosamine galactosyltransferases from several sources are subject to control by α-lactalbumin. In the presence of α-lactalbumin, such enzymes have been shown to catalyse the synthesis of lactose from UDPgalactose and glucose. To test the effect of α-lactalbumin on the purified enzyme from mouse mastocytoma, glucose, UDPgalactose and varying amounts of α-lactalbumin were incubated with the galactosyltransferase. As is evident from Fig. 8, α-lactalbumin greatly stimulated the synthesis of lactose whereas galactosyl transfer to *N*-acetylglucosamine was impeded.



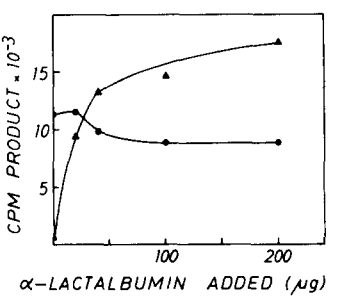


Fig. 7. Heat stability of purified *N*-acetyllactosamine synthetase. The enzyme was pretreated for 3 min at the temperatures indicated, cooled on ice, and assayed for activity under standard conditions (see Fig. 2). The preliminary heat treatment was carried out in the absence of precursors (○), or with UDPgalactose (0.025 μCi; 5 Ci/mole) added (■) or with *N*-acetylglucosamine (1.0 μmole) present (×).

Fig. 8. Stimulation of lactose synthesis (▲) from UDPgalactose (0.05 μCi; 190 Ci/mole) and glucose (2 μmoles) in the presence of purified enzyme (5.5 μg of protein) and α-lactalbumin. Similarly, the effect of α-lactalbumin on the transfer of galactose to *N*-acetylglucosamine (1 μmole) is illustrated (●).

DISCUSSION

The scope of the present investigation has been to study in some detail the *N*-acetyllactosamine synthetase which was recently detected in the microsomal fraction derived from mouse mastocytoma[1].

In order to achieve an adequate purification of the enzyme, two types of gel columns were prepared which were designed to interact specifically with the *N*-acetyllactosamine synthetase. In one approach, *N*-acetylglucosamine was attached to Sepharose 4B with a long hydrocarbon chain interposed between the ligand and the matrix backbone. The importance of placing the ligands at some distance from the solid support in cases which involve ligand-protein interactions of low affinity, has been pointed out[13]. When aliquots of the gel were incubated with UDP[^{14}C]galactose and galactosyltransferase, 30% of the radioactivity became attached to the gel in 60 min, indicating that the covalently linked substrate was a good acceptor for galactose. However, only a partial purification of the enzyme was obtained under the conditions tested, and the yield was modest. It is possible that a highly substituted gel may accomplish a complete adsorption of the enzyme, yielding a more homogenous preparation. To elute the adsorbed enzyme, the alkali procedure described for β-galactosidase by Steers *et al.*[18] is not applicable, since the purified *N*-acetyllactosamine synthetase was unstable at high pH values. Other methods, such as the use of substrate-containing buffers, may effect the elution of an adsorbed enzyme.

For routine preparations of the purified enzyme, chromatography on gels which had been substituted with α-lactalbumin was carried out[14]. In recent years, several studies have appeared which have implicated the A subunit of lactose synthetase with the UDPgalactose: *N*-acetylglucosamine galactosyltransferase present in several tissues[19–22]. Since preliminary experiments indicated that the *N*-acetyllactosamine synthetase from mouse mastocytoma is also subject to control by α-lactalbumin, it is conceivable that a purification of the enzyme based on its affinity for this regulator protein might be achieved, in accordance with the procedure described by Andrews[14] for soluble lactose synthetase A protein from milk. The results presented here indicate that a substantial single step purification of the solubilized enzyme may be accomplished on α-lactalbumin–Sepharose gels, provided that elution is performed in the presence of *N*-acetylglucosamine. With some enzyme preparations, however, the *N*-acetyllactosamine synthetase was eluted slightly behind the main protein peak even in experiments with no *N*-acetylglucosamine included in the eluting buffer. Whether or not this slight retardation was caused by the presence of endogenous *N*-acetylglucosamine moieties in the crude soluble enzyme fraction is unclear.

Solubilization of membrane proteins, particularly with methods such as the rough detergent-alkali procedure employed here, may conceivably result in a soluble but structurally rather heterogenous population of a certain protein with a specific function. The purification of such a protein based on conventional methods may become laborious and impractical. Affinity adsorbents for the purification of such proteins would therefore seem to be the method of choice[23].

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