# GALACTOSYLTRANSFERASE ACTIVITIES IN HUMAN SERA: DETECTION OF A CANCER-ASSOCIATED ISOENZYME

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SUMMARY: Sera from normal subjects and cancer patients were evaluated for galactosyltransferase activity including kinetic and electrophoretic properties. Discontinuous polyacrylamide electrophoresis of whole sera demonstrated the presence of an additional peak of enzyme activity in cancer sera. The majority of recovered enzymatic activity in both normal and cancer sera ran as a broad band of activity behind an albumin standard; the cancer-associated peak was slower moving. Techniques have been developed for the rapid assay of galactosyltransferase activity from gel slices which permit detection of both peaks of activity.

## INTRODUCTION

Galactosyltransferase activity, usually associated with intracellular membranes (1), has been detected as a cell surface enzyme and also as a soluble activity in several biological fluids (2-5). The cell surface membrane-bound, enzyme has been implicated in cell-cell recognition and cell adhesion (6). The soluble galactosyltransferase activity in human serum, amniotic fluid and cerebrospinal fluid, as well as the other biological fluids, has been recently studied (2-5) but the origin and function of these soluble activities are unknown. Kobata et al (7) have suggested that at least one form of the human milk enzyme may be involved in the biosynthesis of blood group substance B. Kim et al, have suggested that a large fraction of the detected human serum activity may be synthesized in the liver (10).

In the present study we have confirmed many of the properties of the serum galactosyltransferase activity described by Kim et al (3). Further-

more, we have examined the electrophoretic characteristics of the enzyme from various human sera on polyacrylamide gel electrophoresis and have found that the majority of the detectable activity runs as a single broad peak (at pH 8.3). In addition we have detected a second, slower moving peak of galactosyltransferase activity in the sera of cancer patients which has not been detected in the sera of normal subjects and a variety of other patients with non-malignant diseases. A detailed analysis of the patient data will be presented elsewhere.

## MATERIALS AND METHODS

UDP-[$^3$H]-galactose (1.18 Ci/mmole) was obtained from New England Nuclear. A stock solution, 727 μM, was prepared to a final specific activity of 0.076 Ci/mmole using unlabeled UDP-galactose obtained from Sigma Chemical Company. Polyacrylamide and bisacrylamide were obtained from Eastman Kodak Company. Reagents were twice-recrystallized from chloroform and acetone respectively prior to use. Glycoprotein acceptor in these studies was fetuin minus terminal sialic acid and penultimate galactose residues (SGF-fetuin), which was prepared as described previously (3). The fetuin acceptor was used as a stock solution containing 50 μmoles of acceptor sites/ml.

Galactosyltransferase activity in serum was measured by modification of the method of Kim et al (3). The assay mixture included 10 μl serum, 15 μl UDP-[$^3$H]-galactose stock solution, 40 μl 0.1 M sodium cacodylate, pH 7.2, and 0.154 M NaCl, 50 μl $H_2O$, 15 μl 0.2 M MnCl and 20 μl of SGF-fetuin stock solution. The mixture (150μl) was incubated for 30 minutes at 37°. The reaction was terminated by addition of 5% phosphotungstic acid (v/v) and 2 M HCl and activity determined as previously described (8).

Polyacrylamide gels (8.0%) were prepared from solutions made fresh as follows: A: 9.4 g Tris, 23 μl Temed (Eastman) and $H_2O$ to 100 ml, pH 8.8, B: 2.76 g solution of acrylamide, 0.071 g Bis-acrylamide, and $H_2O$ to 10 ml, and C: 0.30 g ammonium persulfate (Eastman) and $H_2O$ to 100 ml. Gels were formed in glass columns (5 mm x 75 mm), previously washed with a dilute solution (1:200 v/v) of Photo-Flo (Kodak), using a gel mixture containing 3 parts A, 2 parts B and 2 parts C. Gels were photopolymerized. Samples of serum saturated in sucrose were applied in a volume of 25 μl to which 3 μl of 0.44 M dithiothreitol was added. Upper and lower buffer chambers contained a stock solution (6.0 g Tris, 28.8 g glycine, and $H_2O$ to one liter, pH 8.3) diluted 1:10 v/v prior to use. Electrophoresis was carried out at a constant current of 3 ma/gel for 65 minutes (buffer front 45-50 mm from origin) under constant cooling (4°).

After electrophoresis, gels were sliced into fractions 2.5 mm thick, and each fraction, after quartering, was incubated for 60 minutes at 37° in 150 μl of 0.1 M sodium cacodylate pH 5.3 and 0.5 mM N-acetylglucosamine. Determination of galactosyltransferase from gel fractions was carried out using a modification of the procedure described above. Assay mixtures included: 50 μl sample gel-slice eluant, 15 μl of UDP-[$^3$H]-galactose stock solution, 30 μl 0.14 M Na-cacodylate pH 7.2 and 0.22 M NaCl, 15 μl 0.2 M MnCl and 40 μl of SGF-fetuin stock solution. Incubation at 37° was terminated after 60 minutes and activity determined as above.

## RESULTS AND DISCUSSION

Measurement of galactosyltransferase activity in normal human serum yielded results similar to those of Kim et al (3). Using the conditions described here, measurement of serum from normal controls demonstrated an average activity of 74 μmoles/10 μl serum/30 minutes. Both UDP-galactose and SGF-fetuin were present in optimal concentrations as determined by Lineweaver-Burk plots. The Michaelis constant for UDP-galactose was found to be 11 x $10^{-6}$M, in reasonably good agreement with that reported by Kim et al (3). Incorporation of radioactivity into acid precipitable material was found to be linear for greater than 90 minutes and directly related to the amount of serum added (up to 30 μl of serum). Activity was absolutely dependent on the presence of $MnCl_2$; the optimum concentration was 7.5 - 10 mM. After hydrolysis of the reaction product (8) all incorporated radioactivity was shown to co-chromatograph with genuine galactose in two solvent systems. Measurement of activity in serum from patients with carcinoma of colon, pancreas and stomach yielded a slightly increased ($p < .05$) overall average activity (Table I). The difference in the amount of galactosyltransferase activity in normal and cancer sera did not appear to be the result of hydrolases acting on UDP-galactose or on the acceptor. UDP-galactose pyrophosphatase was found to be equivalent in both normal and cancer sera when determined according to the method of Coleman et al (9) (Table I). In addition, we were unable to detect β-galactosidase activity in serum at the alkaline pH of the galactosyltransferase assay (Table I). While Kim et al observed a single broad optimum at pH 7.0, we found a bimodal relationship with optima at pH 6.3 and pH 7.2 (Fig. 1). Enzyme activity was consistently depressed at approximately pH 6.8.

Subsequent to determination of total galactosyltransferase activity in sera, samples were applied to polyacrylamide gels and electrophoresis was performed as described. As Figure 2 illustrates, the majority of galactosyltransferase activity in normal and cancer sera was detected as a broad, relatively swiftly moving band. The peak of activity ran behind a bovine serum

Table I. Galactosyltransferase, Galactosidase and UDP-Galactose Pyrophosphatase Activity in Human Serum, pH 7.2

| Enzyme | Enzyme activity pmoles/60 min/10 µl serum | |
|---|---|---|
| | Control | Carcinoma |
| Galactosyltransferase* | 148 ± 14 | 208 ± 18 |
| β-Galactosidase† | N.D. | N.D. |
| UDP-Galactose pyrophosphatase†† | a. 13.2 ± 4.0 | 11.9 ± 2.7 |
| | b. 21.8 ± 8.5 | 15.0 ± 7.0 |

Mean ± S.E.

* Galactosyltransferase activity was determined as described in Methods.

† N.D. = not detectable. β-galactosidase was measured in 10 µl of serum using 1.0 ml of p-nitrophenyl-galactosidase as substrate (2.0 mM) Glycosidase activity was determined by measurement of optical absorbance at 400 nm.

†† a. UDP-galactose pyrophosphatase was examined by determination of gal-1-phosphate and free galactose at the end of incubation using descending chromatography systems described by Coleman et al (9).
b. Pyrophosphatase activity was determined as described for (a) but reaction mixtures were adjusted to pH 10.2, incubated with alkaline phosphatase and the amount of radioactive galactose was determined as described by Coleman et al (9).

albumin standard. Kim et al had also detected a broad band of activity on cellulose-acetate electrophoresis (10). A variable amount of enzyme failed to enter the gel and remained at the origin as indicated in the representative electropherograms depicted in Figure 2. In total, approximately 30% of applied activity was accounted for after electrophoresis. In addition, a second, slower moving, peak of activity has been consistently detected in the serum of more than 20 patients with colonic, pancreatic and gastric carcinoma. This represented a relatively small proportion of the total activity obtained after electrophoresis (5-20%). This peak

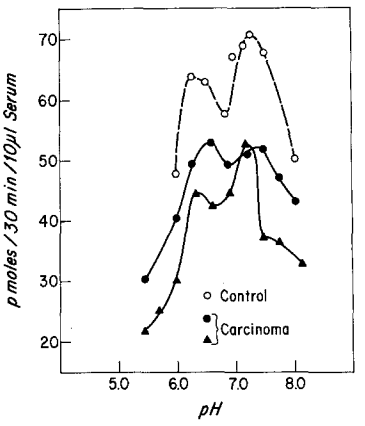


Figure 1. Representative pH curves of human normal and cancer sera.

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has not been detected in the serum of patients with liver disease and normal controls. We were unable to detect the slower moving peak unless gel reagents were first recrystallized; this may be explained by an overall decrease in recovery of galactosyltransferase activity from gels made with unpurified reagents.

Techniques are being developed to increase the yield and sensitivity of the electrophoresis procedure and we are examining sera from a wider variety of patients to determine whether the slower moving isoenzyme is specific for malignant disease and, if so, whether there is any correlation with the extent of the malignancy. Glycosyltransferase enzymes have been presumed to be involved in cell-cell interactions (6). Thus, unusual cell surface glycosyltransferase enzymes may participate in the altered cell-cell interactions seen in malignant cells. Whether the slower moving galactosyltransferase enzyme, described here, represents a soluble form of one of these putative cell surface enzymes has not yet been determined. Alternatively, the presence of an unusual galactosyltransferase in serum may indicate the biosynthesis of altered glycoproteins. This galactosyltransferase isoenzyme may be another example of an onco-fetal factor derived from the cell

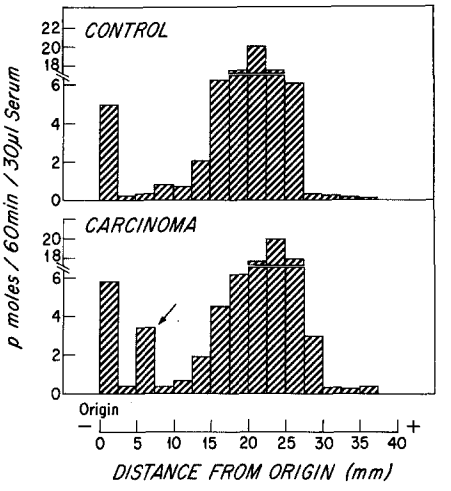


Figure 2. Representative electropherograms of human normal and cancer sera. Conditions of electrophoresis and galactosyltransferase enzyme assay are described under Methods. Arrow points to slower moving peak found in serum of patient with proven carcinoma of the colon.

surface. The results obtained using serum from patients with various malignant diseases and a description of their clinical findings will be presented elsewhere.

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