CHARACTERIZATION OF GLYCOPROTEIN–GALACTOSYLTRANSFERASE ACTIVITY IN ASCITIC FLUID OF BALB/C-YC8 MICE

Evelyne MOREL and Nicole ACHY-SACHOT,

Institut d'Immuno-Biologie de l'INSERM, Association Claude Bernard, Laboratoire associé du CNRS N° 143 and EPHE, Hôpital Broussais, 96 rue Didot, 75674, Paris Cedex, 14, France

and

Geneviève SPIK and Jean MONTREUIL

Laboratoire de Chimie Biologique and Laboratoire associé au CNRS N° 217, Université des Sciences et Techniques de Lille I. B.P. N° 36, 59650, Villeneuve d'Ascq, France

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1. Introduction

Galactosyltransferase activities have been described in various biological fluids [1–5] and more recently in human cancer sera [6], but to our knowledge not yet in cancerous ascitic fluids. So we have undertaken to investigate the galactosyltransferase activity of ascitic fluids towards endogenous and exogenous acceptors, and comparatively in sera from the Balb/c mouse strain bearing the ascitic transplantable isogenic tumor YC8, a lymphoma which possesses at least two tumor membrane antigens [7]. On the other hand, Bosmann and Hilf [8] suggest that during neoplastic growth and tumor invasion, enzymes could diffuse from the tumor site towards the host fluids. If so, their soluble, non-membraneous nature could make their isolation and characterization easier.

For this study a chromatographic method of determining galactosyltransferase activity was applied [9] using ovomucoid as exogenous acceptor. This glycoprotein appeared in our experiments as the best galactose acceptor. It contains five *N*-acetylglucosamino residues in non-reducing terminal positions [10].

2. Materials and methods

2.1. *Preparation of ovomucoid*

Ovomucoid was prepared as described by Jakubczak and Montreuil [11]. UDP-[^{14}C]galactose (specific activity 274 mCi/mM) was purchased from New England Nuclear Corporation. Radioactivity was counted on paper (see section 2.3) in a toluene PPO–POPOP (PPO 5 g, POPOP 0.5 g, Toluene 1 litre) scintillant liquid using Mark I and Intertechnique SL40 liquid scintillation counters.

2.2. *Preparation of tumor cell suspensions*

A strain of YC8 ascite cells was carried in adult Balb/c mice following intraperitoneal injections of 1.0 ml suspension containing 1 to 3 × 10^6 cells. Cell suspensions were harvested weekly. Sera from ascitic mice and from normal Balb/c mice were collected. Normal liquid peritoneal fluid was obtained from standard animals. Cells suspensions were centrifuged at 700 g at 4°C to separate cells and ascitic fluids. Ascitic fluids were further centrifuged (20 000 × *g*) to remove cellular debris.

2.3. *Measurement of enzyme activity*

The complete reaction mixture consisted of 10 μl of biological fluid (ascitic fluid, peritoneal fluid or sera) containing 50 μg of protein, 10 μl of 1 M Tris buffer 7.2, 5 μl of 0.4 M $MnCl_2$, 10 μl of ovomucoid solution as exogenous acceptor (400 μg), 5 μl of UDP-[^{14}C]galactose solution (61 pM and 16.8 nCi). Incubations were carried out at 37°C for 60 min. The reaction was stopped quickly in an ice-bath by the addition of 10 μl of 0.2 M EDTA. Aliquots of each incubation mixture were applied to Whatman No 3 paper sheets and submitted to descending chromatography during 18 h in ethyl acetate/pyridine/acetic acid/water (5:5:1:3; v/v/v/v) according to Spik et al. [9]. In this chromatographic system, the radioactivity of the compound remaining at the origin represents the amount of [^{14}C]galactose incorporated in the endogenous or exogenous glycoprotein acceptors.

2.4. *General methods*

Proteins were determined by a modification of the Lowry method with crystalline bovine albumin as standard [12,13].

3. Results and discussion

Protein amounts were 35 ± 4 mg/ml in ascitic fluid, 2 ± 1 mg/ml in normal peritoneal fluids, 51 ± 5 mg/ml in sera from ascitic mice, and 68 ± 4 mg/ml in normal sera. These results show a hypoproteinemia in sera from ascitic mice.

In preliminary experiments, we have defined some requirements for galactose transfer in YC8 ascitic fluids and sera; the results we obtained are described in table 1. The enzyme shows a requirement for Mn^{2+} which cannot be replaced by Mg^{2+} either in ascitic fluid or in sera. Its activity is greater at 37°C than at 20°C. Optimal pH values for activity are: enzyme from ascitic fluid 6.3 and 7.0; from normal and ascitic sera 6.3 and 7.2 (fig.1). More significant is the decreased activity at pH 6.5 for the ascitic fluid enzyme as compared to pH 7.0 for the serum enzyme. All these values are reproducible and similar results have been recently obtained by Podolsky and Weiser [6] with normal human and cancerous sera.

In table 1 are reported the *exogenous* (transfer to ovomucoid) glycoprotein–galactosyltransferase activities of YC8 mice ascitic fluid and sera, on the one hand, and of normal mouse peritoneal fluid and sera, on the other hand. There is a high galactosyltransferase activity in the ascitic fluid as compared to normal peritoneal fluid. In a similar manner, galactosyltransferase activity is greater in sera from ascitic mice than from normal mice.

The effect of ovomucoid concentration is shown in fig.2: the apparent K_m values for ovomucoid are 1.80×10^{-5} M for the ascitic fluid enzyme and 2.92×10^{-5} M for the normal sera enzyme. An intermediate value of 2.53×10^{-5} was found for the ascitic

Table 1
Exogenous glycoprotein–galactosyltransferase activities[a] in ascitic fluids and sera from Balb/c-YC8 mice

Incubation mixture	Activity (%)	[^{14}C]Gal transferred in (pmol/mg protein/h) × 10^2
Ascitic fluid, complete mixture	100	94 600
Minus ascitic fluid	0.15	142
Minus Mn^{2+}	1.5	1418
Minus Mn^{2+}, plus Mg^{2+}	2.1	1986
Peritoneal fluid	7.8	7378
Serum from ascitic mice complete mixture	100	65 600
Minus serum	0.15	98
Minus Mn^{2+}	1.0	656
Minus Mn^{2+}, plus Mg^{2+}	1.0	656
Normal serum	79.9	52 400

[a]Ovomucoid used as acceptor

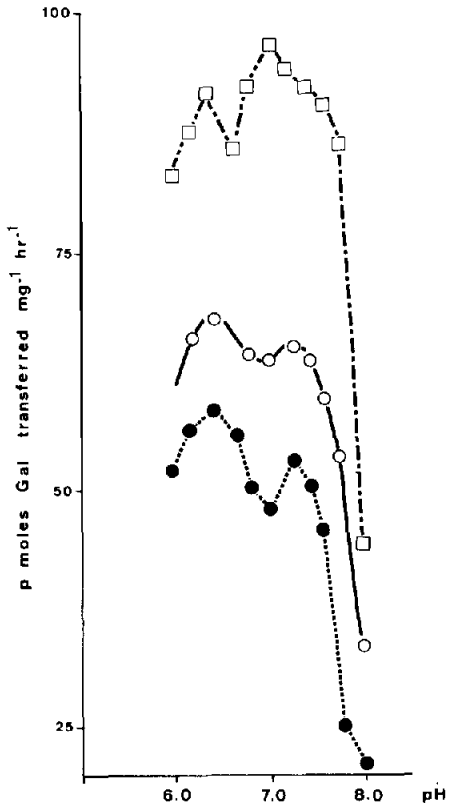

Fig.1. Effect of pH on the galactosyltransferase activity in ascitic fluid (□), sera from ascitic mice (○) and sera from normal mice (●). Incubation conditions are described in Materials and methods.

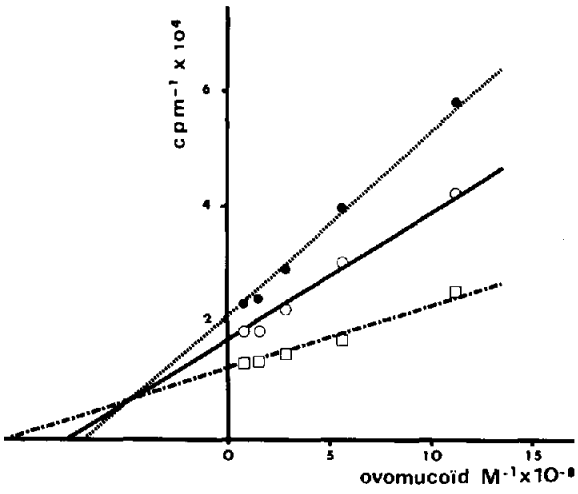

Fig. 2. Lineweaver–Burk plot of the galactosyltransferase activity for ovomucoid by ascitic fluid (□), sera from ascitic mice (○) and sera from normal mice (●). Incubation conditions are described in Materials and methods. Reciprocal values of activity are plotted versus reciprocal values of ovomucoid concentration.

mice sera. The apparent K_m values for UDP-galactose are not significantly different: 1.49, 1.49 and 1.79 (all $\times 10^{-5}$ M) for ascitic fluid, ascitic sera and normal sera, respectively.

The determination of *endogenous* glycoprotein–galactosyltransferase activities (table 2) reveals striking and even more significant differences: enzymatic activity is 80-fold higher in ascitic fluid than in normal peritoneal fluid, and 6-fold higher in YC-8 mice sera than in normal mice sera.

4. Conclusion

The determination of galactosyl transfer activity in presence or absence of ovomucoid leads us to the demonstration of an important glycoprotein–galactosyltransferase activity towards exogenous and endogenous

Table 2
Transfer of galactose to endogenous acceptors in ascitic fluids and sera from ascitic and normal mice

Enzyme from	[^{14}C]Gal transferred in c.p.m./mg protein/h	[^{14}C]Gal transferred in (pmol/mg protein/h) $\times 10^2$
Normal mice sera	232 ± 12	70.4
Ascitic mice sera	1396 ± 84	424
Normal peritoneal fluid	41 ± 14	12.5
Ascitic fluid	3244 ± 136	984

acceptors, in ascitic fluid and in sera from Balb/c-YC8 mice.

The data suggest that the galactosyltransferase of ascitic fluid is different from that of normal sera: the activity as a function of pH and the K_m values for ovomucoid are significantly different. On the other hand, the fact that these parameters are intermediate in the case of the YC-8 mice sera suggest that the ascitic fluid enzyme diffuses from the ascite towards the blood plasma.

The elevated level of galactosyltransferase activity in the ascitic fluid of Balb/c-YC8 mice suggests a diffusion of the enzyme from the cancerous cells to the ascitic fluid, thus supporting the hypothesis of Bosmann and Hilf [8]. However we cannot eliminate, on the one hand, the possibility of the appearance or the increase of enzymic activities, and on the other hand, the appearance of galactosyl-acceptor entities produced by the animal itself in reaction against the tumor. Experiments are now underway to determine the origin of these enzymes and the mechanism of their diffusion.

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