# Comparative interaction of asialoorosomucoid and desialylated ovin submaxillary mucin with hepatocytes from normal and diabetic rats

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## 1. INTRODUCTION

The hepatic binding protein, a mammalian membrane lectin [1], recognizes the serum galactose-terminated desialylated glycoproteins and mediates their uptake and lysosomal degradation [2,3]. The decrease of its biological activity has been reported [4] in streptozotocin-diabetic rat hepatocytes. A dirct correlation has been established between this decrease and the lowering of hepatic lectin content on the surface of diabetic rat hepatocytes [5].

Stockert et al. have shown that the removal of sialic acid from this purified membrane lectin virtually abolishes its capacity to bind galactose-terminated disialylated glycoproteins such as asialoorosomucoid [6]. Since a decrease of the sialic acid content of the hepatic membrane in the diabetic state has been shown by several authors [7,10], this data could explain our previous results.

Whereas the purified desialylated hepatic lectin does not recognize asialoorosomucoid, the desialylated ovine submaxillary mucin, a glycoprotein whose carbohydrate moiety consists almost entirely of *N*-acetylgalactosaminyl residues is recognized by the both sialylated and desialylated hepatic lectin [6,11].

By exploiting this difference in affinity of the hepatic lectin, for galactosyl and *N*-acetylgalactosaminyl-terminating glycoproteins, we have been able to show that the hepatic lectin, present on diabetic hepatocyte membranes, is normally sialylated. So, in diabetes mellitus, the decrease of the hepatic lectin activity is not directly related to the lowering of the sialic acid content of hepatocyte surface glycoproteins.

*Abbreviations:* ASOR, asialoorosomucoid; DOSM, desialylated ovin submaxillary mucin.

## 2. MATERIALS AND METHODS

Human orosomucoid and desialylated ovine submaxillary mucin were kindly supplied by Professeur Montreuil (Université des Sciences et Techniques, Lilles, France) and Professeur Van den Eijnden (Vrije Universiteit, Amsterdam, The Netherlands) respectively. Asialoglycoproteins were labelled as described previously [4]. Their specific activities were about $0.7 \times 10^6$ dpm/$\mu$g. Collagenase was obtained from Worthington. Male Sprague–Dawley rats (275–300 g) were obtained from Charles Rivers (France). Scintillation fluid (aqueous combining system) was from Amersham (France). Other chemicals were reagent grade.

### 2.1. *Rat treatment and cell preparation*

After an overnight fast (with free access to water) rats were randomly divided into two groups. Animals from one group received streptozotocin (65 mg/kg body weight, dissolved in isotonic saline acidified to pH 4.5 with citric acid) through the tail vein (diabetic rats). Rats from control group were injected with the medium alone (normal rats). After 11 days, animals with a blood

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sugar level superior to 250 mg/100 ml were considered as diabetic and used for cell preparation. Hepatocytes were prepared using the collagenase perfusion procedure of Berry and Friend [12]. Final cell pellets were suspended in Krebs buffer containing 0.15 M NaCl, 0.005 M KCl, 0.001 M $KH_2PO_4$, 0.001 M $MgSO_4$, 0.003 M $CaCl_2$, 0.025 M $NaHCO_3$, maintained at pH 7.4 with continuous gassing of $O_2/CO_2$ (95/5). Routinely 70–85% of single cells and 80–90% of viable cells, as judged by either trypan blue exclusion or the release of lactate dehydrogenase (EC 11.1.28) [13] were obtained from normal or diabetic rats.

### 2.2. *Neuraminidase treatment of hepatocytes*

The hepatocytes ($1 \times 10^7$ cells/ml), isolated from normal rats were incubated in Krebs buffer with 100 mU/ml of neuraminidase *Vibrio cholerae* (EC 3.2.1.18, Berhingwerke) for 60 min at 4°C. Following incubation, cells were centrifuged $50 \times g$ for 2 min and washed with 10 volumes of ice-cold Krebs buffer. No modification of cell-viability was observed during the experiment.

### 2.3. *Cell-surface binding*

To measure the surface binding capacity, hepatocytes ($1 \times 10^6$ cells/ml) were suspended in Krebs buffer and incubated for 60 min at 4°C with 4 μg/ml of radiolabelled ASOR or DOSM. As previously reported, this asialoglycoprotein concentration is sufficient to obtain a full saturation of surface receptors [4]. The reaction was stopped by adding 3 ml of ice-cold Krebs buffer into the tubes. Cells were centrifuged $50 \times g$ for 2 min and washed four times in ice-cold Krebs buffer. The cell pellets were dissolved in 10 ml of scintillation fluid and counted in a liquid scintillation spectrometer (Intertechnique SL 30).

Correction for non-specific binding was made by deducing cell associated radioactivity in the absence of calcium, which is a requirement for specific binding to receptor [14,15].

### 2.4. *Total uptake determination*

For determination of total uptake of ASOR or DOSM, hepatocytes ($1 \times 10^6$ cell/ml) were incubated in Krebs buffer containing 4 μg/ml or radiolabelled asialoglycoproteins at 37°C for different times. The cells were kept in suspension in a gyratory shaking bath. Then the reaction was stopped and the cells were washed as described above.

## 3. RESULTS AND DISCUSSION

Neuraminidase treatment of normal rat hepatocytes reduced their capacity to bind and take up ASOR by over 80% and 75% respectively, without affecting DOSM binding and endocytosis (table 1, fig.1). These results agree with those reported by Stockert et al. [11].

As previously reported [4], the binding and the uptake of ASOR by diabetic rat hepatocytes were reduced by half as compared to that of normal rat hepatocytes (table 1, fig.1). We failed to find any discrepancy between the binding and uptake of both DOSM and ASOR in diabetic rat hepatocytes (table 1, fig.1).

Since the neuraminidase treatment of hepatocytes prevents the interaction of hepatic lectin with ASOR but not with DOSM, our results suggest that the decrease of sialic acid content of the hepatic membrane does not account for the changes of hepatic lectin behaviour in the diabetic state. The desialylated hepatic lectin does not occur on the cell surface of diabetic rat hepatocytes.

We have previously reported [4] that the insulin treatment of diabetic rats restores the biological properties of hepatic lectin whereas the withdrawal of insulin-treatment induces again a loss of its activity. The regulatory mechanism of insulin on protein synthesis [16] could account for the decreased content of physiologically active hepatic lectin on diabetic hepatocyte membranes. Another explanation could be modification of the membrane fluidity observed in streptozotocin-diabetic rat hepatocytes. Indeed Nassar et al. [9] have recently reported a significant decrease of the amount of cholesterol and sialic acid, with no appreciable change in the phospholipid content, resulting in a decrease in the cholesterol–phospholipid ratio. Such membrane changes could induce either a defect of the hepatic lectin insertion into the membrane during its turnover or any disruption of the recycling mechanism [16] permitting a reutilization of the hepatic lectin. In the latter case, the amount of intracellular hepatic lectin [17] would not be modified and the effect of insulin treatment of diabetic rats would induce the return to normal membrane fluidity.

Table 1

Surface binding of asialoglycoproteins by isolated rat hepatocytes

| | Surface bound asialoglycoproteins (ng/$10^6$ cells) | |
|---|---|---|
| | Asialoorosomucoid | Desialylated ovin submaxillary mucin |
| Normal rat hepatocytes | 9.8 ± 1.5 | 9 ± 1 |
| Neuraminidase-treated normal rat hepatocytes | 2 ± 0.5 | 8 ± 1 |
| Diabetic rat hepatocytes | 4.2 ± 0.6 | 4 ± 0.8 |

Each value give the mean ± SE from six separate experiments using six rats of each group. All determinations were performed in triplicate.

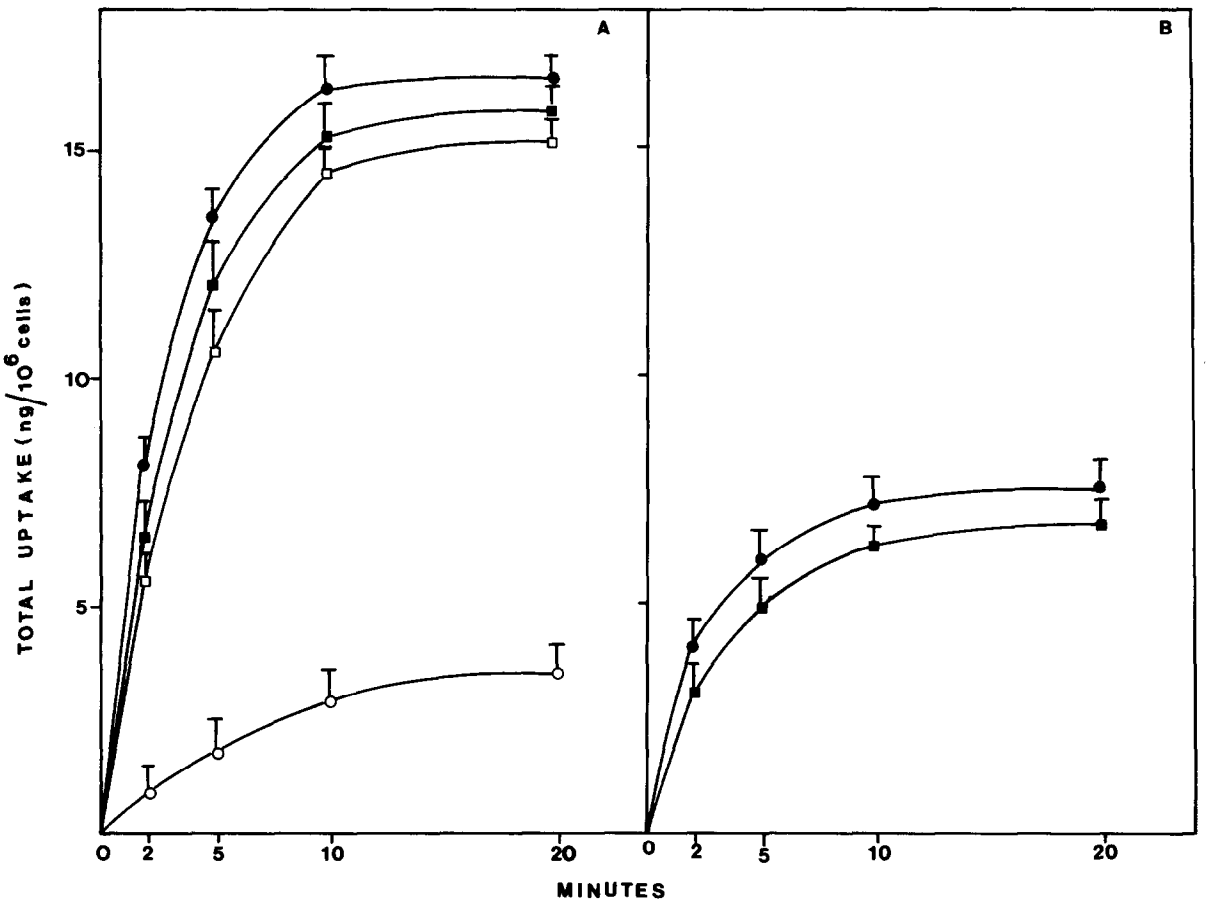


Fig.1. (A) Kinetic of asialoglycoprotein total uptake by isolated rat hepatocytes. (•——•) ASOR total uptake by untreated hepatocytes; (▪——▪) DOSM uptake by untreated hepatocytes; (○——○) ASOR total uptake by neuraminidase-treated rat hepatocytes; (□——□) DOSM total uptake by neuraminidase-treated rat hepatocytes. (B) Kinetic of total uptake of ASOR (•——•) and DOSM (▪——▪) by diabetic rat hepatocytes.

Whatever the hypothesis retained the injury of the hepatic lectin activity in streptozotocin diabetic rat hepatocytes can justify the glycoprotein accumulation observed by several authors in the diabetic rat serum [18].

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