THE EXISTENCE OF A SECOND ROUTE FOR THE TRANSFER OF CERTAIN GLYCOPROTEINS FROM THE CIRCULATION INTO THE LIVER

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SUMMARY

Oligosaccharide chains of agalactoorosomucoid, α_1-acid glycoprotein from which sialic acid and galactose have been sequentially removed, terminate in N-acetylglucosaminyl residues. This protein is rapidly transferred from the circulation into the liver by a route distinct from that previously demonstrated for a number of galactosyl terminating glycoproteins.

INTRODUCTION

The intravascular survival of a number of mammalian plasma glycoproteins is markedly shortened when their terminal sialyl residues are removed (1). To the present it has appeared that these modified proteins disappear rapidly from the circulation and are transferred into the liver only because desialylation has changed the terminal group of the proteins' carbohydrate chains to galactosyl residues that can form a link binding the protein to a specific hepatic receptor (2). We now present evidence for the existence in rats of a second route by which at least one modified glycoprotein, in which N-acetylglucosaminyl residues have been made terminal, is transferred rapidly from the circulation into the liver.

MATERIALS AND METHODS

Orosomucoid or α_1-acid glycoprotein (OR) was isolated from pooled human serum by the procedure of Whitehead and Sammons (3). Asialoorosomucoid (asialo-OR) was prepared by hydrolysis of a

1% solution of OR at 80°C for 1 hour in 0.1N H_2SO_4, followed by exhaustive dialysis against distilled water. The sialic acid released from OR, determined as described by Warren (4), amounted to 98% of the sialic acid originally present.

Agalactoorosomucoid (agalacto-OR) and agalactoceruloplasmin (agalacto-CPN) were prepared by incubation of 10 - 100 mg of the respective desialylated protein with β-galactosidase, from Diplococcus pneumoniae, kindly provided by Dr. Gilbert Ashwell (5). Asialo-OR and agalacto-OR were labelled using the Chloramine-T method (6) with 2 mCi of carrier-free $Na^{125}I$ per 50 μg of protein. The yield of iodinated protein was 85 - 90%, and specific activities ranged from 0.5 to 0.8 μCi per μg.

[^{125}I]-ahexosamino-OR was prepared by incubating 25 μg [^{125}I]-agalacto-OR with 0.37 U β-N-acetylglucosaminidase from Diplococcus pneumoniae, also provided by Dr. Ashwell, in 0.8 ml of 0.25M phosphate-citrate buffer, pH 5.3, containing 0.8 mg of bovine serum albumin (Sigma), for 18 hours at 37°C.

Labelled human asialo-CPN and agalacto-CPN were prepared as described previously (7). Human serum albumin (Sigma) was heat-denatured by the method of Benacerraf et al (8). Total protein was determined by the micro method of Lowry et al (9).

The intravascular survival times of the various glycoproteins studied were determined following injection of 1.0 ml of their solutions in 0.9% sodium chloride into the tail vein of male albino rats weighing 250 - 300 g. Radioactivity was assayed in samples of blood taken from the tail vein at intervals after injection, and of various organs when the rats were sacrificed.

RESULTS AND DISCUSSION

The experimental results shown in Fig. 1 and Table 1 (Exp. Nos. 1 - 4) indicate that asialo-OR and agalacto-OR are transferred from the circulation to the liver through different routes or mechanisms. Fig. 1 shows that when 5.0 μg of [^{125}I]-asialo-OR are infused in the rat intravenously, the physiological half-life of the protein is between 1 and 2 minutes. But the simultaneous infusion of 5 mg of cold asialo-OR prolongs this to 37 minutes, by overloading the route through which removal occurs (1).

Exactly the same effect is seen with agalacto-OR: the half-life of the tracer dose of labelled protein, also about 2 minutes, is lengthened by an order of magnitude when 5 mg of

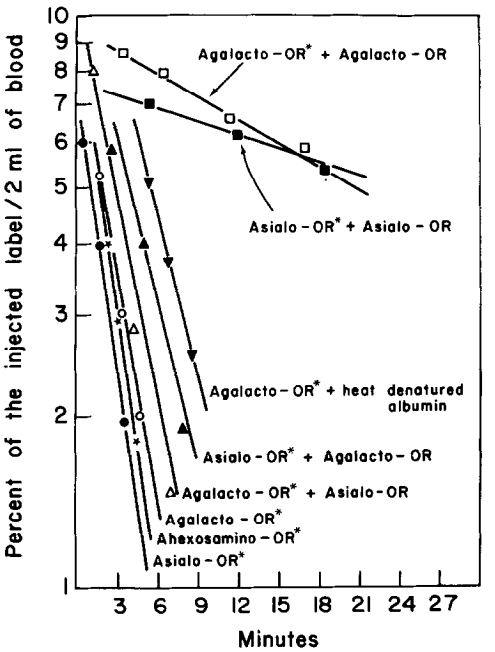

FIGURE 1. Intravascular survival time of modified glycoproteins. All rats were injected with Iodine-125 labelled orosomucoid derivatives (5 μg) with or without addition of an unlabelled glycoprotein (5 mg); or heat denatured albumin (10 mg). Labelled proteins are denoted by *. Lines are drawn by the method of least squares.

TABLE 1

Distribution of radioactivity 15 minutes after injection of 5 μg of labelled modified glycoproteins into rats

Exp. No.	Injected human glycoprotein: Labelled	Unlabelled	Percent of injected label recovered in: Blood	Liver	Kidneys
1	asialo-OR*	asialo-OR (5 mg)	48	19	
2	agalacto-OR*	asialo-OR (5 mg)	3	93	
3	agalacto-OR*	agalacto-OR (5 mg)	35	22	
4	asialo-OR*	agalacto-OR (5 mg)	10	85	
5	asialo-OR*		6	75	4
6	agalacto-OR*		4	77	2
7	asialo-CPN*		2	76	
8	agalacto-CPN*		43	30	
9	ahexosamino-OR*		12	25	42

cold agalacto-OR are infused with the [^{125}I]-agalacto-OR.

However, the infusion of 5 mg of cold agalacto-OR negligibly lengthens the half-life of [^{125}I]-asialo-OR, and 5 mg of cold asialo-OR, in the reciprocal experiment, also affects only insignificantly the half-life of [^{125}I]-agalacto-OR. If there were a common route of removal of asialo-OR and agalacto-OR from the circulation there should also have been prolonged survival of the two labelled proteins in these two experiments comparable to that seen when the same labelled and cold proteins are infused.

The mechanism of removal of agalacto-OR does not involve the reticulo-endothelial system which has previously been shown to play no role in removing a galactosyl terminated glycoprotein, asialo-CPN, from the circulation of rabbits (1). In that study the simultaneous injection of heat denatured albumin, which is known to be taken up by R-E cells (8), did not interfere with the removal of asialo-CPN, and Fig. 1 shows that denatured albumin does not inhibit the disappearance of agalacto-OR, either.

The results of Exp. Nos. 5 - 8 of Table 1 show that the agalacto-derivatives of ceruloplasmin and orosomucoid differ with respect to the duration of their intravascular survival, in contrast to asialo-derivatives of both proteins. Thus, 15 minutes after injection 43% of agalacto-CPN, but only 4% of agalacto-OR, remains in the blood, while asialo-CPN and asialo-OR have disappeared from the circulation and been taken up by the liver to the same extent. These experiments, in rats, confirm quantitatively our previous findings in the rabbit for the modified ceruloplasmins (10).

When sialic acid is removed from orosomucoid, or cerulo-

plasmin, several galactosyl residues are made terminal and this is the structural change previously shown to be responsible for the greatly accelerated transfer of these proteins from the circulation to the liver (1). From mammalian liver we have isolated an hepatic binding protein, HBP, capable of specific binding of asialo-OR, asialo-CPN, and a number of other galactosyl terminating proteins (2). In a number of appropriate in vitro experiments we have been unable to find any evidence of binding of agalacto-OR to HBP, which is consistent with our assumption that the route of removal from the circulation of agalacto-OR by hepatocytes does not involve interaction of this protein with HBP.

Since the carbohydrate chains of agalacto-OR terminate predominantly, if not exclusively, in N-acetylglucosaminyl residues we have tentatively assumed the existence of a second hepatic receptor specific for glycoproteins terminating in these residues. This hypothesis is consistent with the fact that only a small proportion of injected, labelled ahexosamino-OR, a modified glycoprotein thought to have terminal mannosyl residues, is transferred into the liver, and almost half of it is found in the kidneys (Exp. No. 9, Table 1). This last finding suggests that there may be a renal receptor for mannosyl terminating glycoproteins.

In the chicken partially desialylated glycoproteins circulate in high concentrations and these may be the physiological form of plasma proteins in avian species (11). The recent demonstration of an hepatic receptor for N-acetylglucosaminyl terminating glycoproteins in this species supports this idea (12).

We have previously postulated that the desialylation of certain mammalian plasma glycoproteins, and the subsequent, rapid

binding of the resultant galactosyl terminating proteins to HBP, initiate the catabolic phase of the homeostatic mechanism that establishes an equilibrium between the synthesis and degradation of these proteins (1,2,13,14). The findings reported herein suggest that the removal of glycoproteins from the circulation may also be triggered by the appearance of terminal N-acetyl-glucosaminyl -- and perhaps other -- residues and may involve an hepatic receptor distinct from HBP, or receptors in the kidney.

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