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Isolation and some properties of mammalian hepatic membrane lectins

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Membrane lectins were isolated from sheep, goat, and buffalo liver by chromatography on an asialofetuin (ASF)-Sepharose 4B column. The lectins moved as a single protein band in SDS-PAGE with molecular masses of 42, 54 and 50 kDa, respectively, for sheep, goat and buffalo lectins. The molecular masses remained unchanged in 0.2 M 2-mercaptoethanol. As judged from the inhibition of binding of the lectin to ASF gel, the three lectins were β-galactoside-specific. Sheep, goat and buffalo lectins were found to be sialoglycoproteins containing 18.6, 27 and 38.8 mol/mol lectin of neutral hexose, respectively; the corresponding values for the sialic acid content being 5.3, 8.7 and 11.8 mol/mol lectin. Thus goat and buffalo lectins are physico-chemically different from many mammalian hepatic lectins described so far.

Asialoglycoprotein receptor; SDS-PAGE; (Mammalian liver)

1. INTRODUCTION

Mammalian hepatic membrane lectins appear to serve as asialoglycoprotein receptors [1,2] in the hepatic uptake of circulatory asialoglycoproteins prior to their catabolism. They have also been implicated in exocytosis of endocytosed asialoglycoproteins [3] and possibly in specific cellular interaction [4]. Available data on hepatic membrane lectins show that the lectins from different animals vary significantly in physico-chemical properties [4–7]. Further, hepatic membrane lectins from the same animal may exhibit multiple activities, indicating more than one type of lectin [8–10]. Until now, studies on hepatic lectins have been confined primarily to rabbit, rat and human liver [1,2] and the probable occurrence of lectins in other mammalian livers and their molecular and functional properties have remained to be investigated. We therefore report our results on the isolation and characterization of three previously undetermined hepatic lectins, i.e. goat, sheep and buffalo liver lectins. Strikingly, goat and buffalo hepatic lectins were found to be significantly different in molecular morphology from those hitherto described.

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2. MATERIALS AND METHODS

Proteins, enzymes, Sepharose 4B, acrylamide, 2-mercaptoethanol, phenylmethylsulfonyl fluoride and saccharides were purchased from Sigma (St. Louis, MO). Other reagents used were of analytical grade. Protein concentration was measured by a modified Lowry method [5]. Neutral hexose and sialic acid contents of lectins were determined according to Dubois et al. [11] and Warren [12], respectively, using Sigma D-(+)-galactose and *N*-acetylneuraminic acid as standards. SDS-PAGE was carried out according to Laemmli [13].

2.1. *Preparation of affinity media*

A Sepharose 4B gel slurry (60 ml) activated with 18 g solid CNBr according to Cuatrecasas and Anfinsen [14] was treated with fetuin (130 mg) in coupling buffer [0.2 M sodium carbonate buffer (pH 9.2), containing 0.15 M NaCl] and the bound fetuin was desialylated using 0.7 mg neuraminidase in 0.1 M sodium acetate buffer (pH 5.6) at 37°C for 4 h. From the amount of sialic acid liberated, it was estimated that about 1.3 mg fetuin bound per ml settled gel.

2.2. *Measurement of lectin activity*

Lectin activity was assayed by monitoring the interaction of lectin with ASF gel slurry. Lectin (130 μg in 1 ml) was incubated

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with 1 ml gel slurry in 10 mM Tris-HCl buffer (pH 7.5) containing 0.5% Triton X-100, 0.5 M NaCl, 20 mM $CaCl_2$, and 1% BSA for 1 h at 4°C and the bound protein was eluted with 50 mM ammonium acetate buffer (pH 5.1) containing 0.5 M NaCl and 0.5% Triton X-100.

2.3. *Isolation of membrane lectins*

Acetone powder obtained from fresh liver homogenates prepared in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, was solubilized in 10 mM Tris-HCl buffer (pH 7.5) containing 0.5 M NaCl, 0.4 M KCl, 60 mM $CaCl_2$, 1% Triton X-100 and 0.2 mM PMSF. The supernatant (450 ml containing 1 g protein) was loaded on the affinity column equilibrated with the same buffer. The bound protein was eluted with 50 mM ammonium acetate buffer (pH 5.1) containing 0.5 M NaCl and 0.5% Triton X-100. Protein fractions showing lactose-inhibitable binding to ASF-Sepharose were rechromatographed on the affinity column.

3. RESULTS AND DISCUSSION

About 1 mg purified lectin was obtained from 25 g acetone powder of goat, sheep and buffalo liver by chromatography on the asialofetuin-Sepharose 4B column (see fig.1). The elution profiles for sheep and buffalo lectins were similar and hence have been omitted for brevity. Similar yields have been reported for hepatic lectins from human [4] and rat liver [15]. The three purified lectins were essentially free of extraneous proteins; they migrated as a single protein band in SDS-PAGE (see fig.2, inset). The relative mobilities of sheep, goat and buffalo lectins were 0.42, 0.33 and 0.34, respectively, which according to the calibration curve depicted in fig.2, correspond to molecular masses of 42, 54 and 50 kDa (see table 1). These values remained unaltered in the presence of 0.2 M 2-mercaptoethanol, suggesting the absence of interpolypeptide cross-links in SDS-denatured lectins. The experimental uncertainty in measurement of molecular masses by SDS-PAGE was within 10%. Thus the molecular mass of sheep lectin is significantly lower than those found for goat and buffalo lectins.

The sialoglycoprotein nature of the three lectins was studied by measuring the neutral hexose and sialic acid contents of hepatic lectins, the results

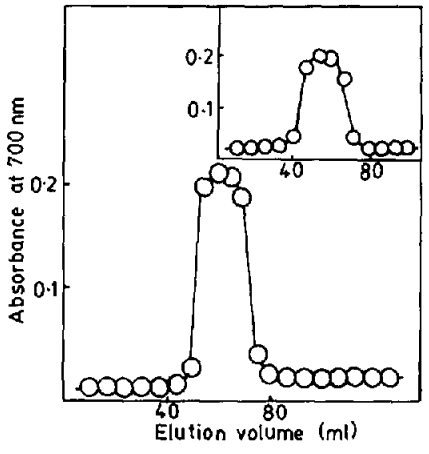

Fig.1. Isolation of goat hepatic lectin by chromatography on an asialofetuin-Sepharose 4B column. 1 g protein in 450 ml of 10 mM Tris-HCl buffer (pH 7.5) containing 0.5 M NaCl, 0.4 M KCl, 60 mM $CaCl_2$, 1% Triton X-100 and 0.2 mM PMSF was applied on an ASF-Sepharose 4B column (2.5 × 12 cm) equilibrated with the same buffer and the bound protein was eluted with 50 mM ammonium acetate buffer (pH 5.1) containing 0.5 M NaCl and 0.5% Triton X-100 in 5-ml fractions at a flow rate of 7 ml/h. Protein fractions were pooled and the pH was raised to 7.5 with 1 M Tris solution. After adding 60 mM $CaCl_2$, the protein solution showing lactose inhibitable binding to ASF-Sepharose was rechromatographed on the affinity column equilibrated with 10 mM Tris-HCl buffer (pH 7.5) containing 0.5 M NaCl, 0.5% Triton X-100 and 60 mM $CaCl_2$. Inset: elution profile obtained on rechromatography of hepatic lectin on the same column.

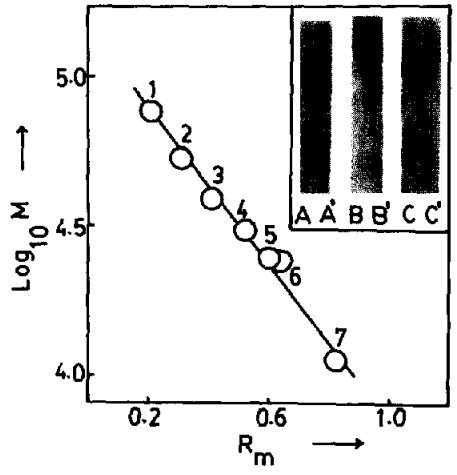

Fig.2. Plot of R_m values of marker proteins vs $\log_{10} M_r$. Marker proteins: (1) bovine serum albumin, M_r 68000; (2) IgG heavy chain, M_r 50000; (3) ovalbumin, M_r 43000; (4) pepsin, M_r 35000; (5) chymotrypsinogen, M_r 25500; (6) IgG light chain, M_r 11700. Inset: electrophoretograms of hepatic membrane lectins of sheep (A,A'), goat (B,B') and buffalo (C,C'), respectively, under reducing (primed letter) and nonreducing (unprimed letter) conditions. 30 μg of each lectin were electrophoresed in 25 mM Tris-192 mM glycine buffer (pH 8.3) containing 0.1% SDS for about 3 h. The slab gel was stained with 1% Coomassie brilliant blue R-250 and destained mechanically with 7% acetic acid.

Table 1

Properties of mammalian hepatic membrane lectins

	Sheep	Goat	Buffalo
(1) Neutral hexose (mol/mol protein)	18.6	27	38.8
(2) Sialic acid (mol/mol protein)	5.3	8.7	11.8
(3) Molecular mass (kDa) under non-reducing conditions	42	54	50
(4) Molecular mass (kDa) in 0.2 M 2-mercaptoethanol	42	54	50

Table 2

Effect of simple sugars on binding of mammalian hepatic membrane lectins to ASF-Sepharose gel

Sugar	Relative inhibitory activity		
	Sheep	Goat	Buffalo
(1) D-(+)-Lactose	1	1	1
(2) D-(+)-Galactose	0.7	0.9	0.8
(3) D-(+)-Fucose	0.03	0.03	0.03
(4) D-(+)-Mannose	0.01	0.02	0.02
(5) D-(+)-Glucose	0.01	0.02	0.02

Relative inhibitory activity: ratio of C_M for lactose to C_M for test sugar where C_M represents the concentration of sugar required for 50% inhibition of binding of lectin to the ASF gel. C_M values for lactose were 12, 15 and 15 mM for sheep, goat and buffalo lectins, respectively

being summarized in table 1. The carbohydrate (hexose and sialic acid) contents of goat and buffalo lectins are markedly higher than those of sheep lectin.

The carbohydrate-binding specificity of sheep, goat and buffalo hepatic lectins was studied by ascertaining the capacity of simple sugars to inhibit binding of the lectins to ASF gel. As shown in table 2 the three lectins appear to be galactose-specific.

From the results presented in tables 1 and 2, it is clear that although the three mammalian hepatic lectins are identical, within experimental error, in carbohydrate-binding specificity, the goat and buffalo lectins are markedly different from sheep lectin in physico-chemical properties. Interestingly, sheep lectin is similar to human [4] and major rat [6] liver lectins in molecular mass and carbohydrate-binding properties. Furthermore, the carbohydrate content (21.4 mol) of human lectin is similar to that (23.9 mol, see table 1) determined for sheep lectin in this study.

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