Affinity chromatography of human serum proteins using matrix bound lectin from *Viscum album* L.

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Summary. The D-galactose specific lectin from *Viscum album* L. reacts with serum proteins that contain the corresponding D-galactopyranosyl residues. By affinity chromatography of human serum on lectin-sepharose IgM, α_2-macroglobulin, haptoglobin and β-lipoprotein were quantitatively retained. Only parts of IgA, IgG and transferrin were retarded. The other serum proteins are unbounded as albumin, β_1A- and β_1C-globulin.

The lectin of the mistletoe, *Viscum album* L., has the property of binding certain carbohydrates and glycoproteins containing D-galactopyranosyl residues[1]. When the lectin is added to human serum, a precipitate is formed composed of the lectin and serum proteins containing the corresponding D-galactopyranosyl moities. Matrix bound *Viscum* lectin should therefore be a valuable tool in serum fractionation, as has been shown for other lectins[2-7]. In the present paper we describe experiments to separate serum glycoproteins with the lectin in a fixed form.

Material and methods. The lectin was prepared from the plant material of *Viscum album* grown on *Robinia pseudoacacia* by affinity chromatography using IgG-sepharose, hydrolized sepharose or D-galactosyl-polyacrylamide as carrier[1,2].

The purified lectin was immobilized by coupling 200 mg to 40 ml sepharose 4B by the cyanogen bromide method[8]. A column of 1.5 × 30 cm packed with the lectin-sepharose was equilibrated in phosphate buffered saline, pH7.2.

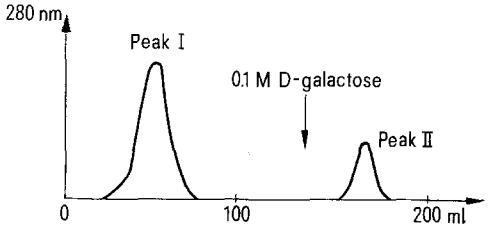
Human serum was dialyzed against this buffer and 2 ml were introduced into the column followed by the buffer. The flow rate was 25 ml/h. The absorption was measured at 280 nm. When a steady baseline had been reached, the displacement of the serum proteins was effected with the same buffer containing 0.1 M D-galactose or lactose. Unadsorbed and desorbed material was pooled and analyzed by immunodiffusion and immunoelectrophoresis. Monospecific immune sera are the products of our institute. Retained and unretained fractions were concentrated to 0.5 ml and so used fore analysis.

Results and discussion. Serum proteins with little or no affinity to the lectin pass through the column unretarded (peak I). But serum proteins which display considerable affinity for the immobilized lectin will be retained. By addition of 0.1 M D-galactose containing PBS, the bound material is eluted (peak II). An indication that the peak I contains serum proteins that fail to interact with the matrix bound lectin and that peak II contains lectin interacting serum proteins is provided by rerunning the pooled dialyzed fraction of peaks I and II, respectively, through the same regenerated column. As before, the material of peak I fails to bind while material of peak II binds to the insolubilized *Viscum album* lectin. This indicates that the failure of peak I to bind and the ability of peak II to bind is not due to overloading of the column or unspecific adsorption, respectively. Analysis by immunodiffusion and immunoelectrophoresis against monospecific immune sera reveals that the first peak contains the serum proteins albumin, IgA, IgG, transferrin, β_1A-globulin and β_1C-globulin, while the second peak contains the serum proteins IgA, IgG, IgM, α_2-macroglobulin, transferrin, haptoglobin and β-lipoprotein (table). Evidently only parts of the proteins IgA, IgG and transferrin are adsorbed on the lectin gel. It is well-known that glycoproteins as serum protein exhibit microheterogeneity with respect to their carbohydrate content, and that the extent of heterogeneity varies from protein to protein[9,10]. Therefore we conclude from our results that the unretained amounts of IgA, IgG, and transferrin differ in their carbohydrate chains from that IgA, IgG, and transferrin that display affinity for the galactose specific *Viscum album* lectin. As yet we do not know whether they differ in the type of glycosidic linkages, in the terminal carbohydrate residue or in sequence of the more internal sugars. This remains to be clarified.

Reaction of immobilized *Viscum album* lectin with serum proteins

Adsorbed proteins	Nonadsorbed proteins
IgA*	IgA
IgG*	IgG
IgM	Transferrin
α_2-Macroglobulin	β_1A-Globulin
Transferrin*	β_1C-Globulin
Haptoglobin	Albumin
β-Lipoprotein	

2 ml human serum was applied to a column of immobilized *Viscum album* lectin (1.5 × 30 cm). Retained proteins were eluted with 0.1 M D-galactose solution. Retained and unretained proteins were analyzed by immunodiffusion against monospecific immune sera. * Denotes that parts of the serum protein react with the lectin.



Affinity chromatography of serum proteins on an insolubilized *Viscum album* lectin column (1.5 × 30 cm). Peak I: unretained serum proteins; peak II: retained serum proteins.

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