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Note

Insoluble D-glucan–glycosaminoglycan complexes formed in aqueous ethanol solution

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Neutral polysaccharides can interact with other macromolecules to form a variety of complexes. Some of these include precipitate formation between dextrans and anti-dextrans[1], D-mannans and D-glucans with[2] a lectin, concanavalin A, and glycogen and alpha-amylase[3]. In addition, neutral polysaccharides can form soluble complexes with such polyelectrolytes as gelatin[4] and bacterial teichoic acids[5] in solutions containing >70% (v/v) of ethanol.

Several studies have shown that D-glucans can form insoluble complexes between RNA and heat-denatured DNA in solutions that contain 20 to 50% of ethanol[6–9]. Graves[7] found that several D-glucans could change the ultraviolet absorption spectra of heat-denatured HeLa cell DNA. Graves *et al.*[6] suggested that D-glucan molecules could play a role in the control of nucleic acid function. The foregoing studies imply that the interactions between D-glucans and nucleic acids are highly specific; however, the only prerequisites for complex-formation appear to be a polysaccharide of high molecular weight, a negative charge on the nucleic acid, and a non-aqueous solvent to lessen water activity, suggesting that any negatively charged polyelectrolyte could form a complex with a neutral polysaccharide. We now show that several glycosaminoglycans can interact with neutral D-glucans to form precipitates in 50% ethanol.

EXPERIMENTAL

Glycogen (Type III, rabbit liver) was a product of Sigma, St. Louis, MO. The dextrans were purchased from Pharmacia, Piscataway, N.J. Heparin (Li salt, A grade) and hyaluronic acid (K salt, human umbilical cord) were obtained from Calbiochem., La Jolla, CA. Dermatan sulfate and chondroitin 4-sulfate were products of Miles Laboratories, Elkhart, IN.

Polysaccharide (1.000 mg) was mixed with polyelectrolyte (500 μg) in a final volume of 4.0 mL containing 50% (v/v) of ethanol. After incubation overnight (~18 h)

at 4°, precipitates were collected by centrifugation (5 min at 18,000 *g*). The precipitates were washed with 50% ethanol (10 mL), evaporated to dryness at 100°, and analyzed for polysaccharide[10] and polyelectrolyte (PE)[11]. In control experiments, PE was not added. The values shown are the net amounts (total *minus* control) of the polymers in the complexes.

TABLE I

PRECIPITATE FORMATION BETWEEN POLYSACCHARIDES AND GLYCOSAMINOGLYCANS IN ETHANOL SOLUTION

Polysaccharide	*Mol. wt.*	D-*Glucan in ppt.* (*μg*)	*Polyelectrolyte (PE)*	*PE in ppt.* (*μg*)
Glycogen		300	Heparin	115
		73	Hyaluronic acid	84
		211	Chondroitin 4-sulfate	188
		196	Dermatan sulfate	89
Dextran	19,900	4	Heparin	0
		7	Hyaluronic acid	0
		3	Chondroitin 4-sulfate	0
		3	Dermatan sulfate	3
	40,000	78	Heparin	29
		47	Hyaluronic acid	25
		63	Chondroitin 4-sulfate	78
		70	Dermatan sulfate	81
	227,000	195	Heparin	126
		83	Hyaluronic acid	59
		164	Chondroitin 4-sulfate	158
		147	Dermatan sulfate	111

RESULTS AND DISCUSSION

The data given in Table I show that the D-glucans (glycogen and two linear dextrans) afford a precipitate with heparin, hyaluronic acid, chondroitin 4-sulfate, and dermatan sulfate in 50% ethanol. In the absence of a D-glucan, all of the polyelectrolytes were completely soluble in the ethanol solution. The dextran of low molecular weight (19,900) failed to give a detectable reaction with any of the glycosaminoglycans; this observation is consistent with the findings of Graves[7], who found that D-glucans of molecular weight $<10{,}000$ fail to interact with nucleic acids. Moreover, Graves[7] showed that D-glucose is not an inhibitor of precipitate formation between nucleic acids and D-glucans. From Table I, it may be seen that the dextran of molecular weight 227,000 precipitates greater amounts of polyelectrolyte than the D-glucan of molecular weight 40,000. These results suggest a cooperative interaction in which the association between the hydroxyl groups of one hexosyl residue and a charged group of the glucosaminoglycan enhances, or stabilizes, additional intermolecular bonding.

The results presented here suggest that the formation of polysaccharide–polyelectrolyte complexes in ethanolic solution, manifested in precipitation, is a general phenomenon. The biological significance of such interactions is probably minimal.

Lindahl and Hook[12] recently reviewed the mechanisms through which glycosaminoglycans are known to interact with biological macromolecules. It is conceivable that polyelectrolytes could complex with carbohydrate-rich macromolecules on hydrophobic areas of membranes. In this regard, we have observed that transforming DNA adheres strongly to the cell wall of *Bacillus subtilis*[13], an interaction supposedly mediated through hydrophobic interactions between the cell wall and DNA-membrane complexes[13].

At the higher concentrations of the reactants, it is possible that a salting-out effect, or an exclusion as described by Laurent[14], occurs. In this kind of interaction, the presence of one type of polymer lowers the solubility of other types.

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