Interaction of Concanavalin A with High Fructose Corn Syrup Glucans and Honey Components

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The interactions of concanavalin A (jack bean lectin) with highly branched glucans isolated from selected samples of high fructose corn syrup were examined turbidimetrically. Polysaccharide mixtures of various lower molecular weight limits were isolated and tested, and glycogen values were shown to increase with molecular weight. As expected, the extent of interaction was dependent on both glucan and concanavalin A concentration, and excess glucan inhibited the interaction. Glucans from conventional corn syrup did not interact with concanavalin A to an extent that could be measured turbidimetrically. Conditions were developed for the isolation of honey retentate whereby it gives no significant turbidity with concanavalin A.

Until recently, typical high fructose corn syrup (HFCS) samples have contained approximately 50% glucose, 42% fructose, and 8% higher saccharides, including some polysaccharide (Mermelstein, 1975). The industrial processes by which a portion of the glucose in conventional corn syrups is enzymatically isomerized to fructose are continually evolving, and presently syrups are available in which the higher saccharide level is greatly reduced and little or no polysaccharide is present.

Our finding that the polysaccharide material from certain HFCS samples is a highly branched glucan suggested that an examination of its interaction with concanavalin A could lead to a test for the presence of HFCS in honey. It has been established that interaction between polysaccharides and concanavalin A depends on the presence of multiple, terminal nonreducing α-D-glucopyranosyl, α-D-mannopyranosyl, or D-fructofuranosyl residues (Goldstein and So, 1965; Goldstein et al., 1965). Precipitation then results from the formation of high molecular weight aggregates of multivalent polysaccharide molecules and tetravalent concanavalin A molecules through the nonreducing terminal units.

Concanavalin A has been intensively examined and its biological and chemical properties reviewed (Lis and Sharon, 1973; Leiner, 1976; Bittiger and Schnebli, 1977). It has a number of activities that make it useful for studying cell surfaces and cell division (Reeke et al., 1974). It is a well-characterized lectin (Edelman et al., 1972; Hardman and Ainsworth, 1972) and in solution at neutral pH is a tetrameric protein with identical subunits, each of molecular weight 26000. The subunits (237 amino acids) have been sequenced, and each contains one Mn^{2+} and one Ca^{2+} binding site as well as one sugar binding site. The cations are essential for sugar binding.

Examination of the interaction of concanavalin A with glycogens, amyloses, and amylopectins from various sources (Cifonelli et al., 1956) revealed a range in glycogen values (G.V., defined in the Experimental Section) from 0.00 to 2.85. The amylaceous samples failed to interact with the lectin, and G.V.'s for glycogen samples increased upon treatment with β-amylase. Later, in an examination of 47 samples of glycogen and amylopectin, Manners and Wright (1962) found that a nearly linear relationship exists between the degree of branching of glycogen-like polysaccharides and their G.V.'s. No interaction was observed between concanavalin A and amylopectin or its limit dextrin. Goldstein et al. (1965), working with highly purified preparations of concanavalin A at higher concentrations, confirmed the importance of polysaccharide branching and showed that, contrary to previous reports, amylopectin does interact to a degree. While all glycogen samples had glycogen values near unity, highly branched yeast mannans (G.V. = 4.96) and bacterial dextrans (G.V.'s to 9.08) had much greater values.

The present study of the interaction of concanavalin A with HFCS glucan and a honey retentate was undertaken to develop a sample screening method for the detection of honey to which HFCS had been added. Several other approaches to screening tests are also under study in this laboratory. A suitable screening method could be used in connection with our confirmatory test based on differing carbon isotope ratios in the two commodities (White and Doner, 1978).

EXPERIMENTAL SECTION

Polysaccharide Isolation. Samples (30 g) of high fructose corn syrup (HFCS) and corn syrup were diluted with 60 mL of deionized water and portions were dialyzed exhaustively against deionized water in tubing (Arthur H. Thomas Co.) with molecular weight cutoffs of 3500, 8000, and 12000; the dialyzate was discarded and the retentate was lyophilized, giving average yields of 0.45, 0.32, and 0.19%, respectively, from HFCS samples and 3–5% from conventional corn syrup samples. HFCS samples were obtained from E. A. Staley Mfg. Co., Decatur, Ill.; CPC International Inc., Argo, Ill.; Clinton Corn Processing Co., Clinton, Iowa; and Corn Sweeteners, Cedar Rapids, Iowa.

Turbidimetric Assay with Concanavalin A. The glycogen value (G.V.) is the ratio of the absorbancy at 420 nm after treatment with concanavalin A of a specified polysaccharide relative to that of a similar amount of purified rabbit liver glycogen (Type V, Sigma). The procedure was a slight modification of that described previously (So and Goldstein, 1967). For the determination, 500 μg of polysaccharide in 1.0 mL of M NaCl containing 0.018 M phosphate buffer (pH 7.2) was added and mixed with 1.3 mg of concanavalin A (Grade IV, lyophylized powder, Sigma) in 2.0 mL of M NaCl containing 0.018 M phosphate buffer (pH 7.2). The tubes were incubated at ambient temperature and the absorbance was measured at 10, 12, and 20 min with a Bausch and Lomb spectronic 20 at 420 nm. G.V.'s were calculated from absorbance at 12 min. Control solutions containing no polysaccharide were prepared in an identical manner. To correct for the turbidity of the polysaccharide solution, 1

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mg of methyl α-D-mannopyranoside was added after the 20-min reading to dissolve the precipitate. The absorbance of this solution was subtracted from the reading obtained before the addition of inhibitor.

Methylation Analysis of HFCS Polysaccharide. Polysaccharide (200 mg) was methylated essentially as described by Sanford and Conrad (1966), wherein the methylsulfinyl anion was used to generate the polysaccharide alkoxide prior to addition of methyl iodide, to obtain 232 mg of product. The methylated sugars constituting the polysaccharide were obtained by acid hydrolysis as described (Sandford and Conrad, 1966) and these sugars were converted to alditol acetates for gas chromatographic analysis (Slonecker, 1972). A sample of maltotriose was methylated and hydrolyzed as above to determine retention times and relative responses of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**) and 1,-4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol (**2**). Likewise, a sample of potato starch was analyzed for degree of branching by this methylation and hydrolysis procedure.

The gas chromatograph was an F&M 810 equipped with a flame ionization detector and contained a stainless steel column (0.125 in. × 14 ft) packed with 3% of OV-17 on Gas-Chrom Q (100–120 mesh). Separations were achieved by isothermal operation at 180 °C for 10 min, then programming at 4 °C/m in to 270 °C. The retention times were: (1), 10.3 min; (2), 15.3 min; and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol (3), 30.3 min. The relative responses were: (1), 4.59; (2), 1.00; and (3), 2.98.

Isolation of Honey Polymers. Honey (30 g) was diluted with 60 mL of deionized water and immediately heated at 85 °C for 30 min to denature and precipitate honey enzymes and protein. It is essential that the diluted samples be heat treated immediately as honey enzymes will degrade polysaccharides in honey and HFCS (Kushnir, 1976). The mixture was then dialyzed exhaustively against deionized water in tubing with a molecular weight cutoff of 12000. The retentate, containing precipitated material, was filtered through Celite and lyophilized. The average yield of solid material from honey was 0.11%. High molecular weight material from honey–HFCS mixtures was isolated as described above for honey, using 12000 cutoff tubing. Certified honey samples representing 1974 and 1975 crops were collected directly from United States beekeepers.

RESULTS AND DISCUSSION

Methylation analysis of an HFCS glucan mixture indicated that it possessed 39% 1→6 branch points. This established the multiplicity of terminal nonreducing α-D-glycopyranosyl residues and prompted the examination of concanavalin A interaction with these glucans. A methylation analysis was also applied to potato starch and a value of 4% 1→6 branches was obtained, corresponding to the literature value (Banks and Greenwood, 1975) and supporting the efficacy of the analysis. The retention times of the three methylated sugar derivatives (**1**, **2**, **3**) from the HFCS glucan were identical with those of the derivatives derived from potato starch, showing that the HFCS glucan is amylaceous, and not dextran.

The sample that contained 39% 1→6 branches gave a G.V. of 2.31. This supports the earlier contention (Manners and Wright, 1962) of the relation between degree of branching and G.V. Their most highly branched glucan, oyster glycogen β-dextrin (17% 1→6 branches) also gave the highest G.V., 1.76.

The glycogen values (G.V.'s) of polysaccharide fractions from HFCS samples are complied in Table I, which includes different HFCS samples obtained from two producers. G.V.'s increased with increasing cutoff of the dialysis tubing from which the polysaccharides were isolated. It is possible that material of molecular weight 3500 to 12000, present in the lower cutoff tubing retentate but not present in the 12000 cutoff retentate, binds to the concanavalin A, but does not form a precipitate. Smith et al. (1968) reported that glycogen samples showed greater turbidity than corresponding samples which had been degraded with β-amylase, amyloglucosidase, acid, or alkali. The opposite effect is seen when high concentrations of concanavalin A are used. Polysaccharides isolated from HFCS samples received from two other producers gave no detectable interaction with concanavalin A, nor did polysaccharide isolated from three samples of conventional corn syrup. As the 12000 cutoff tubing retentates gave rise to the highest glycogen values, all turbidity comparisons here deal with this fraction.

Table I. Glycogen Values of Polysaccharide Fractions from Six Samples of HFCS

	Lower molecular weight limit		
No.	3500	8000	12 000
1	0.59	1.57	2.31
2	1.35	1.92	2.75
3	1.66	2.27	2.97
4	1.23	2.33	2.96
5	1.06	2.03	2.83
6	1.38	2.43	2.89

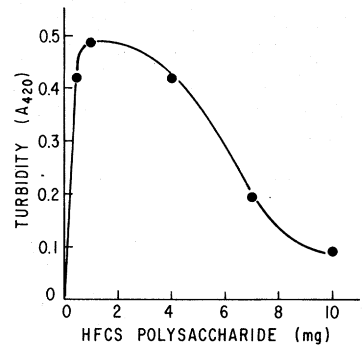

Figure 1. Turbidity with concanavalin A as a function of HFCS polysaccharide concentration. Reaction mixture: 1.3 mg of concanavalin A in 3.0 mL, 10 min.

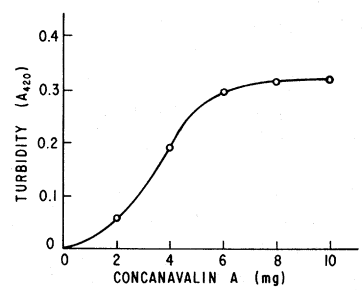

Figure 2. Turbidity with HFCS polysaccharide (>12000 mol wt) as a function of concanavalin A concentration. Reaction mixture: 100 μg of HFCS polysaccharide in 3.0 mL, 10 min.

The interaction of polysaccharide from one sample (**1**, Table I) of HFCS with concanavalin A was examined in detail. This is the polysaccharide that was characterized to contain 39% 1→6 branches (Figures 1–4 all refer to this preparation). An interesting aspect of the concanavalin A–polysaccharide interaction is shown in Figure 1. As the polysaccharide concentration is increased, an equivalence zone is reached where the turbidity no longer increases and further addition of polysaccharide results in solution of the precipitate. As was pointed out earlier (Goldstein et al., 1965), this interaction is analogous in many respects

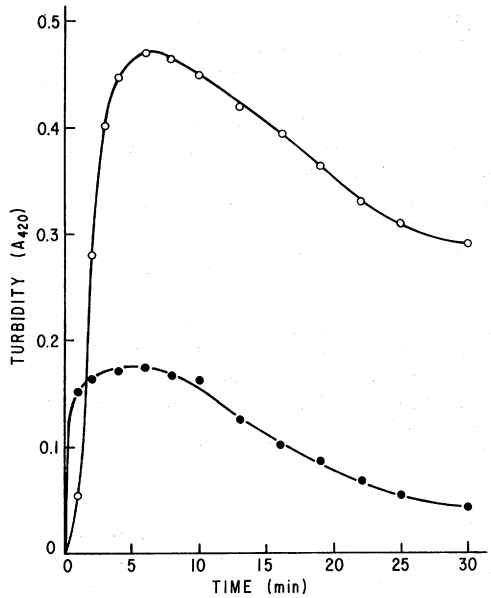

Figure 3. Turbidity of HFCS polysaccharide (○) and rabbit liver glycogen (●) interactions with concanavalin A vs. time. Reaction mixture: 500 μg of polysaccharide and 1.3 mg of concanavalin A in 3.0 mL.

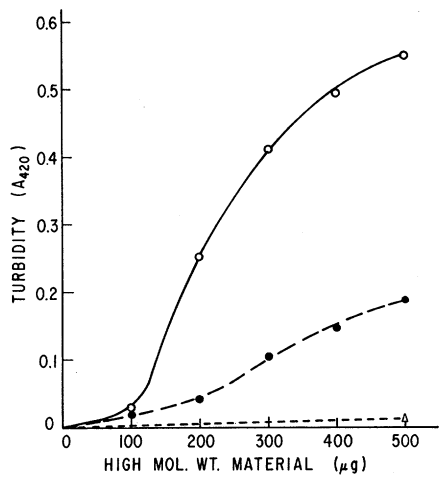

Figure 4. Turbidity as a function of concentration at 10 min for high molecular weight materials with 1.3 mg of concanavalin A in 3.0 mL: (○) HFCS polysaccharide; (●) clover honey retentate; (Δ) clover honey, heat precipitated, then dialyzed and filtered.

to an antigen–antibody system. Common features are specificity, dependence on concentration of both protein and polysaccharide, and ability to be inhibited by low molecular weight compounds (haptens). Another aspect of lectin–polysaccharide interaction is illustrated in Figure 2. When the concentration of concanavalin A is increased and the HFCS polysaccharide level is held constant, the turbidity approaches a maximum value. At lower levels of concanavalin A the turbidity is very sensitive to protein concentration.

The effects of time on the interactions of HFCS polysaccharide and rabbit liver glycogen (the polysaccharide used as a reference in determining G.V.'s) with concanavalin A were compared (Figure 3). Apart from magnitude of turbidity, the polysaccharides behave similarly with time, both having a maximum at 6 min, when the precipitates begin to dissolve. It is obvious that when comparing G.V.'s the protein and polysaccharide concentrations as well as the time of reaction must be precisely controlled. This was emphasized earlier by Poretz and Goldstein (1968), who also demonstrated the effects of hapten inhibitors on the concanavalin A–polysaccharide interaction.

The effects of HFCS polysaccharide concentrations on the interactions with a constant level of concanavalin A are given in Figure 4. The polysaccharide levels are as those in the early portion of the Figure 1 curve, so the zone of equivalence has not yet been reached. Also indicated are the relatively low turbidities displayed by retentate from a clover honey sample and the very slight interaction with concanavalin A of the material isolated from this clover honey sample by dialysis after this honey had been diluted and heat denatured at 85 °C. While the material prepared from dialyzed unheated honey interacted to some extent with the lectin, the material prepared from the heated, dialyzed, and filtered honey was lighter in color and interacted negligibly with concanavalin A. Heat treatment of this honey sample resulted in about a 10% reduction in the yield of retentate. It is probable that glycoprotein, capable of interacting with concanavalin A, was removed from the heat-treated sample. A 50:50 mixture of these HFCS and honey samples was prepared and the turbidity of the freeze-dried retentate with concanavalin A under the standard conditions was 0.242, close to what might be expected. It is essential that such mixtures be heat treated to remove precipitable honey material, as not only will this material interact with the lectin, but active honey enzymes will degrade HFCS polysaccharides, rendering them incapable of interacting with concanavalin A. Polysaccharide from a honey–HFCS mixture, stored 5 months, gave the expected turbidity with concanavalin A, demonstrating that at full density (80–85% solids) no notable enzymatic degradation of HFCS polysaccharides by honey amylases occurs. Again the mixtures should be heat treated immediately upon dilution.

The high molecular weight material from 12 honey samples was assayed for turbidity with concanavalin A, in each case after removal of the heat precipitable material. All preparations gave only a slight interaction with the lectin, with turbidities close to that displayed by the heat-treated sample in Figure 4.

Experiments were conducted to determine if the high molecular weight preparations from honey and HFCS that gave no turbidity with concanavalin A inhibit turbidity formation between the lectin and the G.V. standard, rabbit liver glycogen. No such inhibition was observed, as G.V.'s near unity were obtained when honey and HFCS materials were added.

While amylopectin interacts with concanavalin A (Goldstein and So, 1965), the polysaccharide fraction of conventional corn syrups, derived from corn starch, gives no interaction and that from some HFCS samples, products of corn syrup isomerization, interact significantly. It is possible that during some methods of production of HFCS, processes such as transglucosidation and reversion occur, resulting in the polysaccharides having a greater capacity to interact with concanavalin A. Also, the different enzyme preparations used by the various producers of HFCS will be reflected in the ability of polysaccharide preparations from each sample to interact with concanavalin A.

Differences among high molecular weight fractions of various corn derived syrups can be demonstrated by their differential interactions with concanavalin A. Preparations from some HFCS samples give a pronounced interaction, while those from conventional corn syrups do not interact to an extent that can be detected turbidimetrically. The inability of retentates from some HFCS samples to interact

precludes the use of this approach as a routine screening test for HFCS in honey. Concanavalin A and the host of other plant lectins now available, each with its sugar binding specificity, are of increasing interest. The use of various lectins can provide convenient methods for qualitative analysis, such as described here, and also important tools to be used in both the isolation (affinity chromatography) and structural investigation of polysaccharides.

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