IMMUNOCHEMICAL STUDIES ON DEXTRANS: MICROHETEROGENEITY OF DEXTRAN NRRL B1397 DEMONSTRATED BY PRECIPITATION WITH CONCANAVALIN A

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SUMMARY: Dextran NRRL B1397 containing C-2 and C-3 branching points was fractionated by successive additions of concanavalin A (Con A). Quantitative experiments of the precipitations of the fractionates with Con A and homologous rabbit anti-dextran, and on inhibition of the precipitin reaction showed that the original dextran sample was composed of a heterogeneous population of dextran molecules. This heterogeneity was confirmed by methylation studies. The fractions were found to have almost the same contents of 1→3 branches but different contents of 1→2 branches. Fractions rich in 1→2 branches reacted well with both a homologous anti-dextran serum and Con A, whereas fractions poor in 1→2 branches either reacted little or not at all with the antiserum or Con A.

A dextran produced by Leuconostoc mesenteroides NRRL B1397 was investigated in our laboratories and shown to contain both C-2 and C-3 branching points (1). Furthermore, immunochemical studies indicated that rabbit anti-dextran B1397 sera had at least two specificities, one for α(1→2) and the other for α(1→6) glucosyl glucose linkages (2). Thus this dextran contained at least two kinds of antigenic determinants. This prompted us to examine the distribution of determinants in the dextran molecules, to see whether the dextran sample was composed of antigenically homogeneous molecules.

During these studies on dextrans, dextran B1397 was found to precipitate well with the phytohemagglutinin Con A (3). This protein has high affinity to α-D-mannopyranosyl residues (4, 5), but it also binds to α-D-glucopyranosyl residues (3, 5, 6). It is also known that Con A interacts with dextrans containing many non-(1→6) linkages more readily than with those containing few of these linkages (5, 7). So, we tested whether the dextran B1397 sample could be separated into different populations of molecules with Con A. We tested this with both Con A and Con A-Sepharose and found that Con A was better for this purpose. This paper reports experiments on the fractionation of dextran B1397 by interaction with Con A and chemical and immunochemical analyses of the fractions.

MATERIALS AND METHODS

Dextran NRRL B1397, prepared from L. mesenteroides NRRL B1397, was a generous gift from Meito Sangyo Co., Ltd., Nagoya, Japan. The bacterial strain was kindly provided by Dr. A. R. Jeanes, Northern Regional Research Laboratories, U. S. Department of Agriculture, Peoria, Illinois, U. S. A. The samples of kojibiose (O-α-D-glucopyranosyl-(1→2)-D-glucose) and isomaltose (O-α-D-glucopyranosyl-(1→6)-D-glucose) used were the same as those described previously (8). The anti-dextran serum (156D) and absorbed serum (156D abs.) specific for α-D-glucopyranosyl-(1→2)-D-glucose linkages were described previously (2). Con A was pruchased from Calbiochem, San Diego, California, U. S. A.

Quantitative precipitation and inhibition assays were performed in the usual way (9). Antibody nitrogen in precipitates was determined by the ninhydrin method (10). Quantities of sera containing 3 to 5 μg of precipitable antibody N were used in inhibition assays. Con A-dextran interactions were examined under a modification (11) of the conditions described by So and Goldstein (12). Precipitated nitrogen was determined by the ninhydrin method (10).

Periodate oxidation was carried out by a slight modification of the Smith degradation procedure described previously (13). The dextran sample (about 0.32 μmole glucose) was treated with sodium periodate (1.8 μmoles) at 24°C for 8 days, and then excess periodate was degraded with ethylene glycol (3.6 μmoles). The reaction products were reduced by treatment with sodium borohydride (10 μmoles) at 0°C for 24 hr and the excess borohydride was decomposed with acetic acid. The mixture was dried completely in vacuo over P_2O_5 and NaOH and the residue was treated with 5 % methanolic hydrogen chloride (0.5 ml) at 100°C for 4 hr in a sealed tube. The methanolyzed solution was concentrated to dryness again in a desiccator under a pressure of about 60 mm Hg over P_2O_5 and NaOH, and then the residue was kept overnight in vacuo. The dried material was dissolved in water (1.0 ml) and glucose was determined by the phenol-sulfuric acid method (14). As a control, the same amount of dextran was added to the reagent tube before methanolysis, and the mixture was dried and then methanolyzed. Experimental, control and blank mixtures were set up in triplicate.

Gas liquid chromatography (g.l.c.) was carried out in a Hitachi K-35 chromatograph fitted with a flame-ionization detector and a stainless steel column (4 mm x 2 m). Methylated sugars were analyzed as their corresponding alditol acetates using 3 % ECNSS-M on Gas Chrom Q at 180°C. The peak areas on the gas chromatograms were measured with a Takeda-riken TR 2215A digital integrator.

RESULTS AND DISCUSSION

Fractionation of dextran B1397 with Con A. Dextran B1397 was subjected to fractional precipitation with Con A as follows: dextran (150 mg/140 ml of 1 M NaCl in 0.018 M phosphate buffer, pH 7.2) was fractionated by successive additions of Con A (7.5, 1.5, 1.5, 1.5, 1.5, 1.5, 1.5 and 1.5 ml of solution containing 25 mg of Con A/ml of 1 M NaCl). Each precipitate was washed and dissolved in water by adding excess α-methyl D-mannopyranoside. The resulting solutions and the final supernatant after treatment with Con A were each mixed with a final concentration of 10 % trichloroacetic acid and

centrifuged. The supernatants were extracted with ether and dialyzed against water and then their dextran contents were estimated as glucose (14). The fractions obtained were named A, B, C, D, E, F, G, H and Sup in order of their precipitation. The dextran contents of the fractions were: A 40 mg, B 5.5 mg, C 10 mg, D 8.9 mg, E 9.7 mg, F 6.0 mg, G 2.1 mg, H 0 mg and Sup 1.9 mg. Their total contents of 1→3 linkages were determined by periodate oxidation and the results are given in Table 3.' The fractions had almost the same contents of 1→3 linkages (5 to 7 %).

Quantitative precipitation of dextran fractions with Con A and anti-dextran serum. Quantitative precipitation experiments were performed with Con A and anti-dextran serum to examine the reactivities of the fractions. The results are shown in Fig. 1. The precipitation curves of fractions A, B and C and the original dextran were very similar, whereas those of fractions D, E and F were somewhat different, precipitations being inhibited in the dextran-excess region. However, the maximum precipitations observed with all these fractions were very similar. In contrast, fraction G was precipitated much less and its precipitation curve was quite different from those of the other fractions. Fraction Sup did not give any precipitate. With unabsorbed anti-dextran serum, fraction A gave an identical precipitin curve to that of the original dextran. However, the precipitating abilities of the fractions decreased in the order of their fractionation. Fraction Sup also gave no

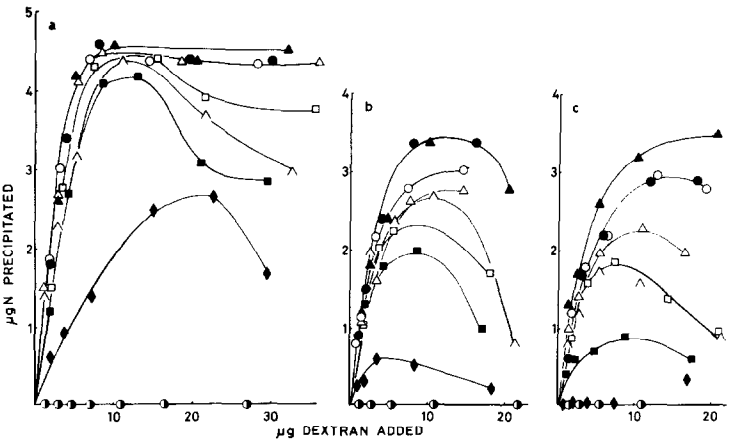

Figure 1. Quantitative Precipitation Curves of Dextran Fractions with Con A and Anti-dextran Sera

a, with Con A (50 μg); b, with unabsorbed serum (5 μl); c, with absorbed serum (8 μl); ●, original dextran; ▲, fraction A; ○, fraction B; △, fraction C; □, fraction D; ∧, fraction E; ■, fraction F; ◆, fraction G; ◑, fraction Sup.

precipitate with anti-dextran under the conditions used. With absorbed antiserum, fraction A reacted more strongly than the original dextran, whereas fraction B gave almost the same curve as the original dextran. A similar decrease in precipitating ability with progress of fractionation was also observed with absorbed antiserum, and with this both fraction G and fraction Sup gave no precipitate.

Quantitative inhibition of precipitations. To compare the antigenic properties of the dextran fractions, quantitative precipitation inhibition experiments were carried out with the original dextran and fractions A, E, F and G, which each gave a different precipitin curve with the antiserum. The dextran was previously shown to have two specificities, so the two disaccharides kojibiose and isomaltose were used as inhibitors. The results are shown in Table 1. Isomaltose was less inhibitory than kojibiose with fraction A, but its inhibitory effect increased in order with fractions E, F and G. Thus, the specificities of the precipitations differed with different dextran fractions. The precipitations of absorbed serum with all the antigens tested were inhibited much more by kojibiose than by isomaltose, as seen in Table 1, indicating that the specificities of the precipitations were almost equally directed to the $\alpha(1\rightarrow2)$ linkage.

Methylation of the fractions. Dried samples of the original dextran and fractions A, E, G and Sup (1 to 3 mg, each) were dissolved in dimethyl sulfoxide (0.5 ml) under a nitrogen atmosphere and treated with methyl sulfinyl carbanion (0.3 to 0.5 ml) (15) at room temperature for 1 hr with stirring in an ultrasonic apparatus. The resulting polysaccharide alkoxides were then treated with methyl iodide (0.5 ml) as described by Hakomori (16).

Table 1. Inhibitions by Two Disaccharides of Precipitation of Dextran Fractions with Anti-dextran Sera

Dextran fraction	With unabsorbed serum		With absorbed serum	
	Kojibiose	Isomaltose	Kojibiose	Isomaltose
Original	1*	1.8*	1*	11.1*
A	1	1.9	1	13.4
E	1	0.71	1	13.6
F	1	0.27	1	12.5
G	1	0.12		

* Relative amount of disaccharide inhibitor required for 50 % inhibition.

The reaction mixtures were dialyzed, and concentrated to a small volume, and the permethylated products were extracted from them with chloroform. The extracts were evaporated to dryness and the residues were hydrolyzed in screwcapped serum bottles by heating first with 90 % formic acid (0.5 ml) at

Table 2. Molar Ratios of Methyl Sugar Components in the Methylated Dextran Fractions

O-Methyl glucose	2,3,4,6-Tetra-	2,4,6-Tri-	2,3,4-Tri-	Mixture of 3,4- and 2,4-di-
Linkage indicated	Glcpl→	→3Glcpl→	→6Glcpl→	→6Glcpl→, →6Glcpl→ (↑2, ↑3)
Dextran: original	1.00	0.05	3.39	0.88
A	1.00	0.06	2.80	0.88
E	1.00	0.03	5.96	0.83
G	1.00	-*	7.79	0.81
Sup	1.00	-*	6.25	0.82

* Small samples were methylated, so the peak of 2,4,6-tri-O-methyl glucitol acetate was not detectable.

Table 3. Percentages of 1→2 and 1→3 Linkages in the Dextran Fractions

Fraction	Branch point[a]	1→3 Linkage[b]	1→3 Branch point[c]	1→2 Branch point[d]
	%	%	%	%
Original	18.38	6.64	5.72	12.66
A	20.57	7.18	5.59	14.62
B		6.20		
C		5.46		
D		5.06		
E	12.52	5.33	4.95	7.57
F		5.44		
G	10.22	5.98	5.98	4.24
Sup	12.12	6.67	6.67	5.45

a Calculated from the quantity of non-reducing terminal groups obtained by methylation analysis.
b Glucose residues resistant to Smith degradation.
c Calculated as (b - percentage of linear 1→3 linkages).
d Calculated as (a - c).

100°C for 3 hr and then with 1 M trifluoroacetic acid (0.5 ml) for 12 hr. After removal of the volatile acid by repeated addition of water and evaporation, the mixture of methylated monosaccharides in the hydrolysate was reduced with sodium borohydride, and converted to alditol acetates by heating with pyridine and acetic anhydride (1:1, v/v, 0.2 ml) at 100°C for 1 hr. Excess reagent was evaporated off and the residue was dissolved in chloroform and subjected to g.l.c. analysis (Table 2). Under the conditions used 2,4-di and 3,4-dimethyl glucose derivatives were not separated, so the content of 1→3 branch points was calculated by subtracting the content of linear 1→3 linkages from that of total 1→3 linkages, as shown in Table 3. The content of 1→2 branch points was calculated by subtracting the content of 1→3 branches from that of total branches. As seen in Table 3, the content of 1→2 branch points was high in fraction A (14.62 %), intermediate in fraction E (7.57 %) and low in fractions G and Sup (4.24 and 5.45 %), whereas the contents of 1→3 branch points were similar in these fractions.

Thus the fractions of dextran B1397 obtained by successive additions of Con A varied in antigenic properties and in their contents of 1→2 branch points, but had similar contents of 1→3 branch points. The sum of the yields of the individual fraction (84.1 mg) from 150 mg of starting material was low, even when loss during experimental procedures is taken into consideration. Both 1→2 and 1→3 branchings were found in all the fractions, but there is no evidence that both these branchings were present in the same dextran molecule. Fractionation of the dextran sample with α(1→2) specific antibody should provide information on this problem. In the present study dextran B1397 was separated into several fractions. These fractions were shown to differ in their contents of 1→2 linkages and in their abilities to precipitate with Con A and with anti-dextran serum. The heterogeneous properties of some soluble dextrans have been reported (17-19). It will be interesting to investigate the relationships between the structures and molecular sizes of the dextrans and the locations of the antigenic determinants in their molecules.

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