IMMUNOCHEMICAL STUDIES ON DEXTRANS: DISTRIBUTION OF α1→2 AND α1→6 SPECIFIC DETERMINANTS OF DEXTRAN NRRL B1397

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Received September 21,1976

SUMMARY: A rabbit anti-dextran serum was separated into two fractions, specific for α1→2 and α1→6 glucose linkages, respectively. Dextran NRRL B1397 containing α1→2 and α1→6 specific determinants was fractionated by step-wise precipitation with the α1→2 specific antibody fraction to see whether the α1→2 and α1→6 specific determinants were both in the same molecule. Most of the dextran fractions were shown to have two specificities by quantitative inhibition experiments with kojibiose and isomaltose as inhibitors. The precipitin lines in agar of dextran fractions with antibody fractions specific to α1→2 and α1→6 fused. These results strongly suggest that these two antigenic determinants are present in the same molecule.

Chemical studies on the highly branched dextran NRRL B1397 have indicated that this dextran has C-2 and C-3 branching points; the branches at C-2 are mainly single D-glucose units and those at C-3 are composed of more than two glucose units (1). Anti-dextran sera prepared by injecting Leuconostoc mesenteroides NRRL B1397 into rabbits had two specificities directed to α1→2 and α1→6 glucose linkages, respectively (2). Recently, we fractionated this dextran by interaction with concanavalin A (3). Several fractions were obtained which all had similar contents of C-3 branches but different contents of C-2 branches. Fractions rich in C-2 branches reacted well with both concanavalin A and a homologous anti-dextran serum, whereas fractions poor in C-2 branches reacted slightly or not at all with concanavalin A or the antiserum. Inhibition experiments showed that the fractions had both α1→2 and α1→6 specificity. However, we did not determine whether the α1→2 and α1→6 specific determinants were both present in the same dextran molecule.

In the present study we fractionated this dextran by step-wise addition of α1→2 specific antibody, to examine the distribution of determinants in the dextran molecules, and whether both determinants are present in the same molecule. This paper reports experiments on the preparation of the α1→2 specific antiboty fraction, fractionation of dextran B1397 by interaction

with the specific antibody and chemical and immunochemical analyses of the fractions.

MATERIALS AND METHODS

Dextran B1397 prepared from Leuconostoc 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. Dextran B512 was also supplied by Dr. A. R. Jeanes. Dextran 2000 was a commercial product from Pharmacia (Uppsala, Sweden). Kojibiose (O-α-D-glucopyranosyl-(1→2)-D-glucose), isomaltose (O-α-D-glucopyranosyl-(1→6)-D-glucose) and isomaltooligosaccharides up to heptaose were the preparations described previously (4, 5). Methyl α-D-glucopyranoside was a commercial product from Pierce (Rockford, Illinois, U. S. A.).

Rabbits were injected with L. mesenteroides NRRL B1397 suspension as described previously (2). Seven days after the last injection, blood was taken and antisera were prepared by the usual method. The two sera obtained (199D and 204D) were combined (Pool 1). Anti B1397 (156D) (2) was also used.

Quantitative precipitation and inhibition assays were performed in the usual way (6). Antibody nitrogen in the specific precipitates was determined by the ninhydrin method (7). Generally, quantities of serum which gave 3 to 5 μg of precipitable antibody N were used for inhibition assays. The precipitin reaction in agar was carried out by Ouchterlony's double diffusion technique with Bacto Special Agar Noble (8).

Periodate oxidation and methylation studies were performed as described previously (3). Sugar was measured by the phenol-sulfuric acid method (9).

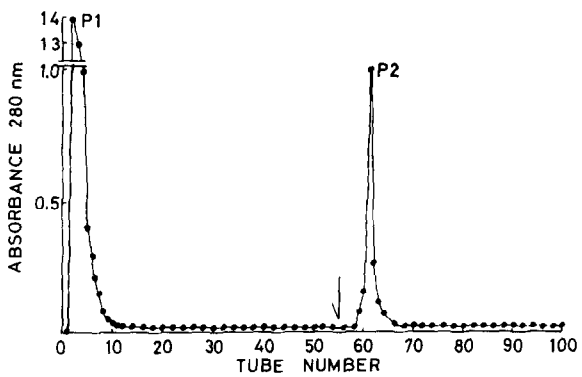


Figure 1. Fractionation of rabbit anti-dextran pool 1 into two distinct fractions. The arrow indicates change from sodium azide-saline to 1 M glucose solution in sodium azide-saline for elution. Fractions of 20 ml were collected at a flow rate of 30 ml per hour.

RESULTS

Fractionation of Anti-dextran B1397 (Pool 1). Anti-dextran Pool 1 (20 ml) was dialyzed against sodium azide-saline (NaN_3 0.02 %, NaCl 0.9 %) and applied to a column of Sephadex G75 (2 x 30 cm). The column was eluted first with sodium azide-saline (1.1 l), and then with 1 M glucose solution in

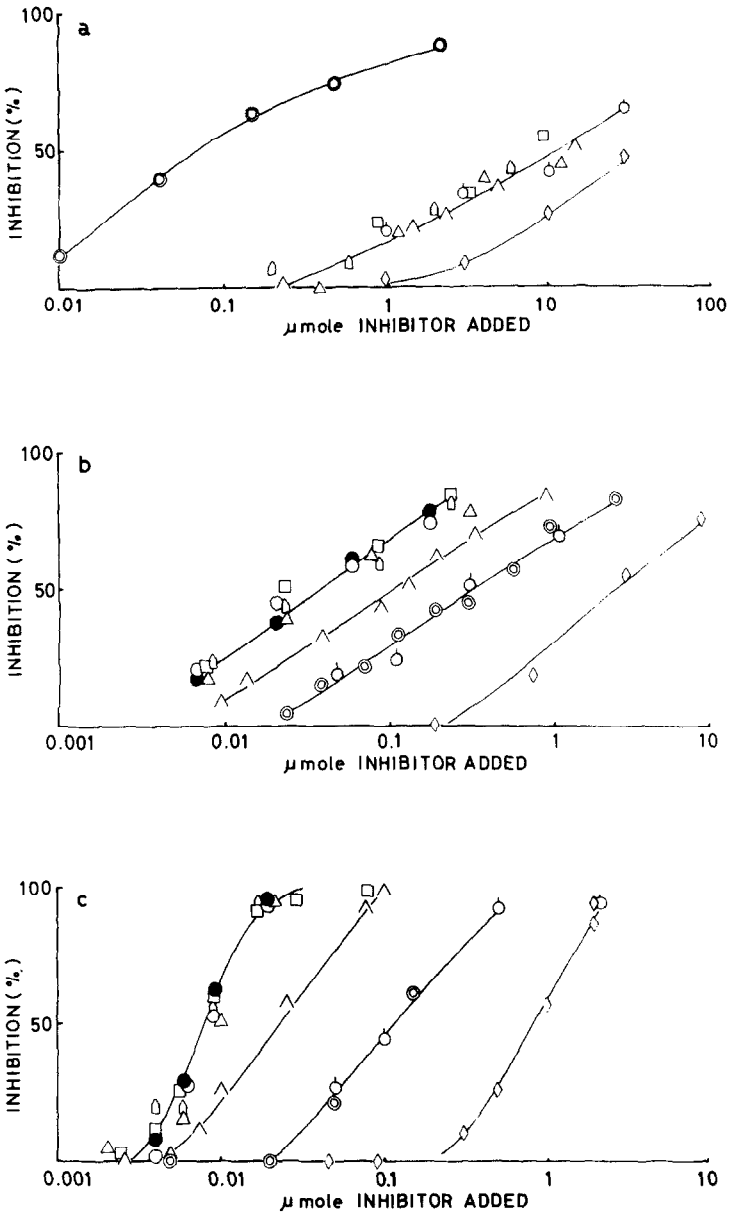


Figure 2. Inhibition by oligosaccharides of precipitation of fraction P1 and P2 by dextrans B1397 and B512. a, P1 and B1397; b, P2 and B1397; c, P2 and B512; ◎, kojibiose; ◇, glucose; ∧, isomaltose; △, isomaltotriose; □, isomaltotetraose; ⬠, isomaltopentaose; ○, isomaltohexaose; ●, isomaltoheptaose; ⬡, α-methyl D-glucopyranoside.

sodium azide-saline (0.9 l). As shown in Fig. 1, two peaks of material (P1 and P2) were eluted. The very small amount of P2 contaminating fraction P1 was removed by chromatography on the same column. Fraction P2 was shown not to be contaminated with P1. On rechromatography of fractions P1 and P2 separately only single peaks of P1 and P2, respectively, were obtained

suggesting that these fractions contained different materials. The fractions containing these materials were each adjusted to 20 ml, dialyzed against sodium azide-saline and stored.

Serological Specificities of Fractions P1 and P2. Quantitative precipitation experiments showed that dextran B1397 reacted well with both fraction P1 and P2. However, dextran B512 which has only α1→6 specificity (10, 11) only precipitated with fraction P2. Quantitative precipitation inhibition assays were carried out to determine the serological specificities of the two fractions. As seen in Fig. 2, precipitation of dextran B1397 with P1 was greatly inhibited by kojibiose, indicating that it showed α1→2 specificity. The precipitations of dextrans B1397 and B512 with P2 were both more strongly inhibited by isomaltose and isomaltooligosaccharides than by kojibiose, showing that P2 had α1→6 specificity.

Fractionation of Dextran B1397 by Precipitation with Specific Antibody Fraction P1. Dextran B1397 (100 mg/50 ml of saline) was precipitated stepwise by successive incubation with increasing amounts of fraction P1 (7, 14, 14, 14, 14 and 7 ml) for 6 to 8 days each at 4°C. Each precipitate was separated by centrifugation, dissolved in water by adding excess 1 M glucose-saline and treated with 10 % trichloroacetic acid. The final supernatant after the serial precipitations was also treated with trichloroacetic acid. The trichloroacetic acid-treated mixtures were centrifuged, and the supernatants were shaken with ether and dialyzed against water. The dialyzed fractions were named A, B, C, D, E, F and the Sup in order of their precipitation. The dextran contents of the fractions estimated by glucose determination were: A 9.1 mg, B 26.6 mg, C 7.2 mg, D 7.2 mg, E 2.6 mg, F 0.5 mg and Sup 3.2 mg.

Periodate Oxidation and Methylation of the Fractions. The contents of 1→2 and 1→3 branching points in the fractions were determined by periodate oxidation and methylation analyses using the methods described previously (3). The results are shown in Tables 1 and 2. Under the conditions used for gas chromatography 2,4-dimethyl 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. 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 2, the content of 1→2 branch points was high in fraction A (26.29 %), intermediate in fractions B, C and D (9.70, 8.34 and 7.40 %, respectively) and low in the Sup (1.78 %), whereas the contents of 1→3 branch points were similar in all the fractions (4.48 to 5.95 %) except the Sup (9.97 %).

Table 1. Molar Ratios of Methyl Sugar Components in 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	Glcp→	→3Glcpl→	→6Glcpl→	→6Glcpl→, →6Glcpl→ ↑2 ↑3
Dextran: Original	1.00	0.05	3.39	0.88
A	1.00	0.10	1.15	0.96
B	1.00	0.05	4.77	0.93
C	1.00	0.03	4.97	1.15
D	1.00	0.03	5.99	0.93
Sup	1.00	0.07	6.44	0.97

Table 2. 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	30.77	7.56	4.48	26.29
B	14.66	5.69	4.96	9.70
C	14.29	6.38	5.95	8.34
D	12.47	5.44	5.07	7.40
E		5.56		
F		7.64		
Sup	11.75	10.79	9.97	1.78

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

Quantitative Precipitation of Dextran Fractions with Serum Fractions P1 and P2. Quantitative precipitation experiments were performed to examine the reactivities of the fractions with serum fractions P1 and P2. The results are shown in Fig. 3. P1 reacted more with fraction A than with the original dextran, and slightly less with fraction B than with the original dextran.

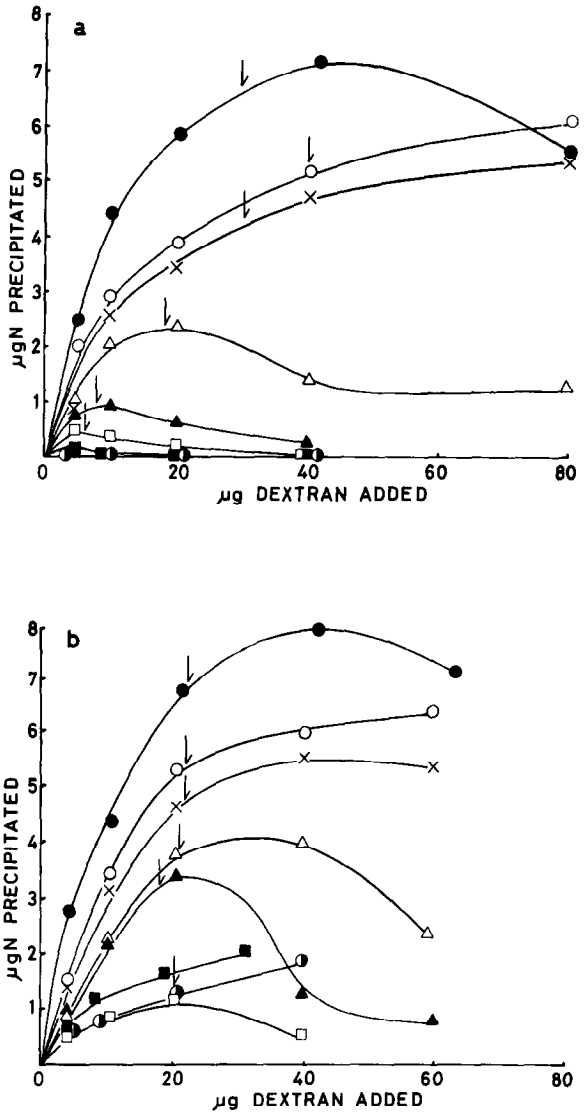


Figure 3. Quantitative precipitin curves of serum fractions P1 and P2 by dextran fractions. a, P1 (20 μl); b, P2 (14 μl); ○, original; ●, fraction A; ×, fraction B; △, fraction C; ▲, fraction D; □, fraction E; ■, fraction F; ◑, fraction Sup.

The precipitating abilities of fractions C, D, E and F decreased in this order and the final fraction Sup did not give any precipitate, as seen in Fig. 3a. With P2, the precipitation curves of fractions A, B, C, D and E were similar with those with P1, whereas those of fraction F and the Sup were different. The Sup precipitated with P2 although it did not with P1.

Quantitative Inhibition of Precipitation. To characterize the specificities of the precipitations of the dextran fractions with P1 and P2,

Table 3. Inhibition by Two Disaccharides of Precipitation of Dextran Fractions with Two Specific Serum Fractions P1 and P2

Dextran fraction	With α1→2 specific P1		With α1→6 specific P2	
	Kojibiose	Isomaltose	Kojibiose	Isomaltose
Original	1[a]	153[a]	1[a]	0.34[a]
A	1	318	1	0.32
B	1	78	1	0.37
C	1	82	1	0.31
D	1	63	1	0.21
E	1	97		
Sup			1	0.22

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

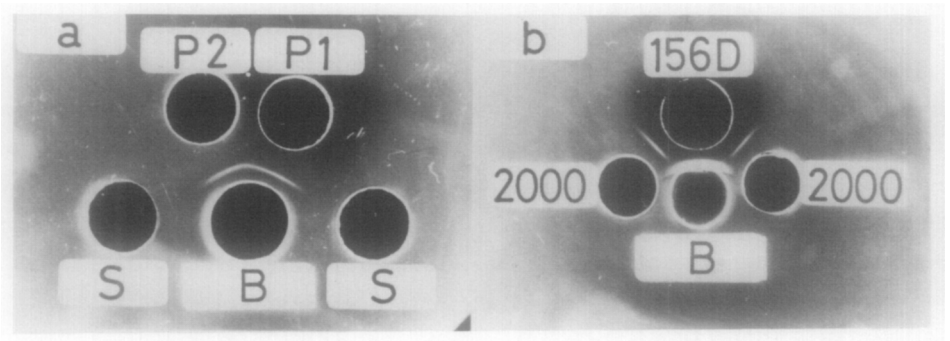


Figure 4. Precipitin reaction in agar. P1 and P2, serum fractions P1 and P2, respectively; 156D, anti-dextran B1397; B, dextran fraction B; S, saline; 2000, dextran 2000.

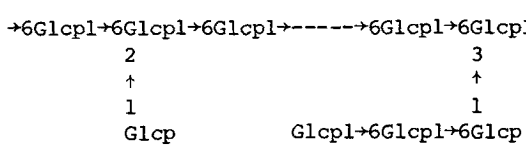
quantitative precipitation inhibition experiments were carried out at the points indicated by arrows in Fig. 3. The dextran was previously shown to have two specificities (2), so the two disaccharides kojibiose and isomaltose were used as inhibitors. As seen in Table 3, with P1, kojibiose was much more inhibitory than isomaltose in all the systems, indicating that all the fractions had antigenic determinants specific for the α1→2 linkage. With P2, isomaltose was a stronger inhibitor than kojibiose indicating that the fractions contained determinants specific for the α1→6 linkage. The fact that the four fractions A, B, C and D, which were specifically separated by precipitation with α1→2 antibodies, had antigenic determinants specific for

the α1→6 linkage suggests that the dextran molecules in these fractions had two distinct antigenic determinants in the same molecule. Precipitation by the double diffusion technique was performed to confirm this.

Precipitation Reaction in Agar. The results obtained with fraction B and dextran 2000 are shown in Fig. 4. The latter dextran was derived from dextran B512, which is known to have α1→6 specificity (10, 11). The precipitin line between B and P1 completely fused with the line between B and P2 (Fig. 4a). The line between B and antiserum (156D) fused with the line between dextran 2000 and the antiserum with a spur (Fig. 4b). Similar results were obtained with other fractions A, C and D. These results indicate that the two antigenic determinants are both in the same molecule.

DISCUSSION

The following structure has been proposed for dextran NRRL B1397 (1):



The side chains joined by α1→2 linkages, which occupy three quarters of the branches, are mostly, if not all, one unit long, whereas those joined by α1→3 linkages may be longer than two residues, and probably consist of three D-glucose residues. Thus 1→2 side chains are apparently responsible for α1→2 specificity, and 1→3 side chains for α1→6 specificity.

Previously we separated this dextran into several fractions by interaction with concanavalin A (3). These fractions were found to differ in their contents of C-2 branching points and also in their serological properties. These findings indicated that the dextran sample was composed of a microheterogeneous population. The present study showed the heterogeneous nature of this dextran more clearly. Fraction A which precipitated on the first addition of α1→2 specific antibody fraction, P1, contained the highest content (26.29 %) of 1→2 branches and the Sup which did not precipitate with P1 contained only a very few (1.78 %) 1→2 branches. However, all the fractions had similar contents (4.48 to 5.95 %) of 1→3 branches, except the Sup (9.97 %). The latter results on 1→3 branches coincide well with previous findings (3).

Fractions A to D, which precipitated with α1→2 specific antibody, had specificities for α1→2 and α1→6, as shown by inhibition experiments. So, the molecules of dextran in fractions A to D each seem to contain two distinct antigenic determinants. This was confirmed by double diffusion precipitation experiments, as shown in Fig. 4.

The precipitation curves of the dextran fractions with P1, which was used for fractionation of the dextran sample were as expected. However, those with P2 were unexpected (Fig. 3): fraction A which precipitated most easily with P1 and contained the highest content of α1→2 branches also reacted most with P2, and fractions B, C, D and E, which showed decreasing reactivities with P1 in this order, also showed the same order of reactivities with P2. The reason for these results in unknown. Fraction F and the Sup which reacted slightly or not at all with P1 gave unique precipitin curves with P2. Further studies were not possible because of the limited amounts of samples available.

The precipitations of B1397 and B512 with P2 were both inhibited most and equally by isomaltotriose, -tetraose, -pentaose, -hexaose and -heptaose (Fig. 2bc). This suggests that the maximum size of the 1→6 specific antibody combining site of P2 is complementary to isomaltotriose. Elution of 1→6 antibodies whose combining sites were complementary to larger oligosaccharides than triose has been reported (12). So, it is possible that 1→6 specific antibodies with binding sites complementary to such large structures, if any, were not eluted with 1 M glucose solution, although antibodies which precipitated with B1397 were mostly recovered in both fraction P1 and P2.

Heterogeneous populations of native dextrans have been demonstrated by alcohol precipitation, boric acid interaction and concanavalin A-Sepharose chromatography (13-15). However, the antigenicities of these dextran fractions were not studied. In the previous and present work the micro-heterogeneous nature of dextran B1397 was studied in relation to the antigenic properties. It would be interesting to know why and how such heterogeneous polysaccharides are produced by microbes, and the role of these heterogeneous biopolymers.

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