Immunochemical Similarity of Synthetic α-(1→3)- Branched D-Glucans to Dextran B1375

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Abstract Anti-dextran B1375 antibodies were raised in rabbits by injecting formalin-killed Leuconostoc mesenteroides strain NRRL B1375, and the anti-dextran serum was used to examine native dextran B1375, and synthetic linear and four α-(1→3)-branched α-(1→6)-D-glucopyranans for similarities. The antiserum reacted with the homologous dextran B1375 and also with all the synthetic linear and branched glucans. Precipitation and precipitation-inhibition studies indicated that the antiserum contained at least three groups of antibodies with different specificities, the first specific for linear α-(1→6)-D-glucopyranan structure, the second specific for α-D-glycopyranosyl-(1→3)-branching and the last specific for another, unknown structure present in the dextran B1375 molecule. Two samples of the synthetic branched glucans were shown to be immunochemically the most similar to natural dextran B1375 by inhibition experiments.

Linear glucans, α-(1→6)-D-glucopyranans, and α-(1→3)-branched glucans, 3-O-[α-D-glucopyranosyl]-(1→6)-α-D-glucopyranans, have been chemically synthesized as natural dextran models (7, 16, 23, 24). Immunological properties of synthetic linear glucans have also been studied (4, 5, 14, 22). These synthetic linear glucans have been shown to precipitate with anti-dextrans B512 serum (14) and N4 (22) or mouse myeloma protein with anti-dextran activity (4). Synthetic α-(1→3)-branched glucans also precipitates with anti-dextran N4 serum, the antibody reacting with the linear backbone of the branched molecule (21).

Abbott et al studied dextran B1375 (Birmingham) and showed that the dextran contains about 15.7% α-(1→3)-branches and that the branches consist mainly, if not exclusively, of single glucose units (1). It is conceivable that the α-(1→3)-branched glucans recently synthesized are similar to dextran B1375. To determine the immunochemical similarity of synthetic branched glucans to B1375 dextran, rabbit anti-dextran B1375 serum was prepared and the reactions of the antiserum
with the homologous natural dextran and heterologous synthetic glucans were compared.

MATERIALS AND METHODS

Synthetic polysaccharides, dextran and oligosaccharides. Synthetic 3-O-[(α-6)-glucopyranosyl]-{(1→6)-α-D-glucopyranans were described previously (7). Samples V39, V17, V37, and V32 were reported to contain 11-12%, 33-43%, 50-54%, and 71-100% branches, respectively. Synthetic linear (1→6)-α-D-glucopyranan (G55), with a molecular weight of 55,000, has also been described (24). Dextran B1375 was a generous gift from Dr. M.E. Slodki, Northern Regional Research Center, Peoria, Ill., U.S.A. Dextran T40 is a commercial product of Pharmacia Fine Chemicals, AB, Uppsala, Sweden. Isomalto-oligosaccharides up to the tetramer, nigerose (αDGlcp(1→6)DGlc) and two nigerosyl trisaccharides, αDGlcp(1→3)αDGlcp(1→6)DGlc and αDGlcp(1→3)[αDGlcp(1→6)]DGlc, were also described previously (17, 20).

Antisera. Rabbit anti-dextran B1375 sera were prepared as follows: Leuconostoc mesenteroides NRRL B1375, generously supplied by Dr. A.R. Jeanes, Northern Regional Research Center, was cultured in medium C as described by Jeanes (8). The cells were killed with 0.5% formalin-saline, washed with sterile saline and suspended in saline (about 40 mg of wet cells/ml). Rabbits (209D and 210D) were immunized by injection into the ear vein of, successively, 0.5, 0.5, 1.0, 1.0, 2.0, 2.0, 3.0, 3.0, 4.0, and 4.0 ml of the suspension twice a week, and blood was taken from the ear vein 8 days after the last injection, for preparation of antisera by the usual method. The precipitable antibody content of sera 209D1 and 210D1 was 0.8 and 1.1 mg of N/ml, respectively. Antiserum 210D1 was used for most of the experiments.

Absorbed antiserum. Antiserum 210D1 (2 ml) was incubated with 200 μg of linear glucan G55 at 37 C for 1 hr and at 4 C for 5 days. The mixture was centrifuged and the supernatant was used as the absorbed antiserum.

Serological assays. The precipitin reaction in agar was carried out by Ouchterlony’s double diffusion technique (12) with Bacto Special Agar Noble (Difco Laboratories, Detroit, Mich., U.S.A.). Quantitative precipitation and precipitation-inhibition assays were carried out in the usual way (9). Antibody nitrogen in the specific precipitates was determined by the ninhydrin method (18).

RESULTS

Precipitation in Agar

Homologous and heterologous reactions were first performed in agar. As shown in Fig. 1A, dextran B1375 reacted most strongly with anti-B1375, four branched glucans, V39, V17, V37, and V32, reacted moderately, and linear glucan G55 weakly. Precipitation lines produced by the four branched glucans all fused with each other and spurs were observed between B1375 and V32 or G55, and also between G55 and V39. These results indicate that at least three specificities were present.
in the anti-B1375 serum, the first one specific for the linear (1→6)-α-D-glucan structure, the second one specific for the α(1→3)-branched glucan structure, and the last one specific for another, unknown structure which might be present in the native dextran B1375.

To remove α(1→6)-specific antibodies the antiserum was absorbed with G55. The absorbed antiserum no longer reacted with G55, and it reacted weakly with V39, strongly with V17, V37, and V32 and most strongly with B1375 (Fig. 1B). A spur between B1375 and V32 was still clearly observed.

**Quantitative Precipitation Reaction**

Quantitative precipitation experiments were performed to compare quantitatively the abilities of the samples to precipitate antibodies from the unabsorbed and absorbed anti-B1375 sera.

With the unabsorbed antiserum, native dextran B1375 reacted most strongly, V37, V17, V39, and V32 reacted moderately, and G55 and T40 reacted weakly (Fig. 2A). With the absorbed antiserum, again B1375 reacted most strongly and V37, V17, V39, and V32 moderately, but T40 reacted very weakly and G55 not at all (Fig. 2B). After removal of α(1→6)-specific antibody by absorption, the precipitating ability of V39 was greatly reduced. The reactivity of V17, V37, or V32 was also reduced.

**Quantitative Precipitation Inhibition**

To determine the specificity of the absorbed antiserum, quantitative precipitation inhibition experiments were carried out with various glucooligosaccharides. The results are shown in Figs. 3 and 4. In the system of precipitation by B1375, isomaltose, isomaltotriose and isomaltotetraose were equally more potent inhibitors than glucose. Nigeroside was more potent than the isomaltooligosaccharides, and the two nigerosyl trisaccharides were equally the most potent one (Fig. 3). Similar inhibition patterns were observed in the V39 and V17 precipitation systems as seen in Fig. 4, A and B. In the V37 and V32 precipitation systems the inhibition patterns were different from the former three. Thus, nigeroside and the two nigerosyl trisaccharides were equally the most potent inhibitors (Fig. 4, C and D).
Fig. 2. Quantitative precipitation of natural dextran B1375 and synthetic linear and branched glucans. A, with unabsorbed serum 210D1 (10 µl); B, with absorbed serum 210D1 (12.5 µl). Total volume of reaction mixture was 250 µl. The experiments were carried out in the usual way (9). ◆, B1375; ▲, V39; □, V17; △, V37; ■, V32; ●, T40; ○, G55.

Fig. 3. Inhibition by various oligosaccharides of precipitation of absorbed 210D1 serum (5 µl) and dextran B1375 (4 µg). Total volume was 260 µl. The experiment was carried out in the usual way (10). ◇, d-glucose; ∧, isomaltose; △, isomaltotriose; □, isomaltotetraose; ◆, nigerose; ▼, αGlcp(1→3)αGlcp(1→6)Glc; ▽, αGlcp(1→3) [αGlcp(1→6)]Glc.

Fig. 4. Inhibition by various oligosaccharides of precipitation of absorbed 210D1 serum and branched dextrans. The quantities of antiserum and antigens and total volume are given in the figures. Symbols are the same as those in Fig. 3.
Fig. 4

A.

Branched glucan V39 4μg
210 D1 abs. 25 μl
Total vol. 340 μl

INHIBITION (%)

0.001 0.01 0.1 1 10

INHIBITOR ADDED (μ mole)

0 20 40 60 80 100

B.

Branched glucan V17 2.5μg
210 D1 abs. 15 μl
Total vol. 285 μl

C.

Branched glucan V37 3μg
210 D1 abs. X4 70 μl
Total vol. 300 μl

D.

Branched glucan V32 3μg
210 D1 abs. 20 μl
Total vol. 310 μl

Fig. 4
DISCUSSION

The recent development of the polymerization technique for carbohydrates made it possible to synthesize stereoregular polysaccharides and the polysaccharides thus obtained have been used as valuable tools for studies on the biochemical and immunological properties of polysaccharides (3–6, 13–15, 19). In the previous study, we showed that the antisera produced by injecting rabbits with N4 dextran-concanavalin A conjugate contained anti-dextran antibody directed to the internal chain of the linear structure of (1→6)-α-D-glucopyranosyl linkages (22). Synthetic α-(1→3)-branched glucans were also shown to react with the above antibody, indicating that the linear backbone regions of the branched molecules reacted with the antibody (21).

A study was carried out on the immunochemical properties of branches of the synthetic α-(1→3)-branched glucans, as reported in this communication. The fact that the branched glucans reacted with rabbit anti-dextran B1375 indicates that the synthetic branched glucans and dextran B1375 contain antigenically similar structures. One of them must be a linear α-(1→6)-D-glucopyranose linkage, because on precipitation in agar both B1375 and branched glucan V39 produced precipitin lines that fused with that of linear glucan G55 although a spur was observed (Fig. 1).

Many anti-dextran sera have been reported to contain antibodies directed to the α-(1→6)-D-glucopyranose chain and antibodies specific for the individual dextran that was used as the immunogen (2, 11).

Since the antiserum used in this study contained antibodies specific for the linear structure, they were removed by absorption with G55. The absorbed serum precipitated with homologous B1375 and also with heterologous branched glucans V39, V17, V37, and V32 (Figs. 1 and 2), indicating that these branched glucans must have immunological identity with B1375. An examination of the identity by precipitation inhibition using glucose oligosaccharides showed the importance of branching units. With B1375, V39, and V17, two trisaccharides, α-DGlcp(1→3)α-DGlcp(1→6)DGlc and α-DGlcp(1→3)[α-DGlcp(1→6)]DGlc, were equally the strongest inhibitors, nigerose was the second strongest inhibitor, and isomaltooligosaccharides were weaker inhibitors (Figs. 3 and 4). With V37 and V32, the above two trisaccharides and nigerose were equally the strongest inhibitors and again isomaltooligosaccharides were weak (Fig. 4). These results indicate that the nigerose structure, both of the branching unit and branching base, is very important as an antigenic determinant, and also suggest that V39 and V17 are more similar to B1375 immunochemically than V37 and V32 are.

The quantitative precipitation experiments with absorbed antiserum show that the most highly branched glucan V32 (71–100%) and those with the fewest branches V39 (11–12%), T40 (5%), and G55 (linear) are less or the least active in precipitation (Fig. 2B). This indicates that the most important antibodies in the precipitation system might have combining sites that fit an adjacent unbranched chain unit and branched chain unit. This interpretation seems to be consistent with the inhibi-
tion studies (Fig. 4). The more highly branched the glucan the more effective nigerose is as an inhibitor relative to nigerose trisaccharides. Apparently some antibodies interact only with the (1→3) linked disaccharide unit while others react more effectively with (1→6) (1→3) trisaccharide units.

REFERENCES


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