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Relationship between Chemical Structure and Anti-Complementary Activity of Plant Polysaccharides*

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It is known that complement system plays the important role in the host defense system, inflammation or allergic reactions, and that activation of the complement system occurs via both the classical and alternative pathways. The classical pathway is activated by an immune complex containing IgM and IgG antibodies, the acute phase protein, C-reactive protein and RNA tumor viruses. The alternative pathway does not require antibodies and is directly activated by polysaccharides, certain immunoglobulins, viruses, fungi, bacteria, certain animal cells and parasites. The common denominator of these activators is still unknown, although carbohydrate has been found to be common constituent of most of them.

In order to clarify the relationship between the chemical structure of the polysaccharide and its anti-complementary activity, the anti-complementary activity of several kinds of plant polysaccharides that had already been characterized by Tomoda *et al.*, were measured. The present paper deals with relationship between polysaccharide structure and anti-complementary activity and with mode of the action of the active polysaccharides. Tests were carried out on anti-complementary activity of various plant polysaccharide including glucomannans, arabinan, and four types of acidic heteroglycans.

Zizyphus-arabinan, paniculatan and plantago-mucilage A were found to have potent anti-complementary activity which was almost the same level as that of the positive control, which was comprised of AR-arabinogalactan mixture from *A. acutiloba* KITAGAWA. Moreover, Abelmoschus-mucilage M, Althaea-mucilage O, Althaea-mucilage R, and Althaea-mucilage OL were shown to have weak anti-complementary activity. However, Zizyphus-pectin A and all of the glucomannans did not show any significant anti-complementary activity. Although Abelmoschus-mucilage G was isolated from *Melvaceae* plant, this polysaccharide did not show any activity either. A significant degree of anti-complementary activity was found in the polysaccharides Zizyphus-arabinan, paniculatan, and Plantago-mucilage A, and these were then assayed for anti-complementary activity in the same experimental system, and paniculatan was found to have the

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highest activity. These anti-complementary polysaccharides were shown to be highly branched structure.

NHS was incubated with paniculatan, Plantago-mucilage A or Zizyphus-arabinan in GVB^{++}at 30° for 30 min, and the residual activity of C 4 was estimated by hemolytic assay. Paniculatan decreased C 4 content of NHS drastically. When NHS incubated with 100 μg/ml of paniculatan was used for C 4 titration, about 50% of the hemolytic titer of C 4 was consumed. The consumption of C 4 by the action of paniculatan was very similar to that of AAF-IIb-3, an anti-complementary polysaccharide from *A. princeps* PAMP. Zizyphus-arabinan and Plantago-mucilage A also decreased C 4 content of NHS significantly. The anti-complementary activity caused by these polysaccharides also diminished in the absence of Ca^{2+} ion with surplus Mg^{2+} ion. In the case of paniculatan, the activity was completely reduced. These results show that the classical pathway of the complement was activated by these polysaccharides. Furthermore when these polysaccharides were incubated with NHS in Mg^{++}-EGTA-GVB^{--} at 37° for 30 min, and a hemolytic assay (ACH_{50}) was carried out using rabbit erythrocytes, Plantago-mucilage A and Zizyphus-arabinan showed anti-complementary activity on ACH_{50} (ACP activity) does dependently. Therefore crossed immunoelectrophoresis was carried out after the incubation of NHS with these polysaccharides in Mg^{++}-EGTA-GVB^{--} to determine whether C 3 activation had occurred. A cleavage of the C 3 precipitin line was obtained in the serum treated with these polysaccharides. Potent ACP-active Plantago-mucilage A caused the highest C 3 cleavage in serum in three kinds of polysaccharides. However, although paniculatan showed potent anti-complementary activity via the classical pathway it also caused a slight C 3 cleavage in serum. These results indicate that Zizyphus-arabinan and Plantago-mucilage A also activate the complement via the alternative pathway whereas paniculatan mainly activate the complement via the classical pathway.

It was suggested that the anti-complementary activity depends on the detailed chemical structure of the polysaccharide. Therefore we tested anti-complementary activity in 17 kinds of plant water-soluble polysaccharides with different chemical structures, and potent anti-complementary activity was observed in three : paniculatan, Plantago-mucilage A and Zizyphus-arabinan. These polysaccharides differed from each other in component sugars, molecular weight, optical rotation and in their acetyl content although they had high branching structures in commonly.

These results indicate that their high branching structure could be involved in the anti-complementary activity, and some structural moiety such as the negative charge may be decide the activation pathway. Further correlation of structure and activity will require more detailed study of the physicochemical properties of anti-complementary polysaccharides.