Inhibitory Effect of Alginic Acids on Hyaluronidase and on Histamine Release from Mast Cells

Masahiro Asada, Makiko Sugie, Mami Inoue, Kazuya Nakagomi, Seiji Hongo,* Katsumi Murata,** Shinji Irie,** Toshio Takeuchi,** Noboru Tomizuka, and Syuichi Oka†

National Institute of Bioscience and Human-Technology, 1-1 Higashi, Tsukuba, Ibaraki 305, Japan
*Toya Medical and Pharmaceutical University, 2630 Sugitani, Toyama, Toyama 930-01, Japan
**Kibun Food Chemifta Co., Ltd., 1-11-8 Shinsayama, Sayama, Saitama 350-13, Japan

Received October 2, 1996

The effects of various types of alginic acid consisting of l-guluronic acids (G) and D-mannuronic acids (M) on hyaluronidase and mast cell degradation were examined. Alginic acid with an M/G ratio of 1.0 exhibited the strongest inhibition of both activities, the higher molecular weight alginic acids of 150 to 370 kDa being preferable in both cases. Esterification of the carboxyl residue enhanced the latter activity.

Key words: alginic acid; uronic acid; inhibitor; hyaluronidase; mast cell degranulation

Alginic acid is in the extra-cellular matrix and cell membrane of brown algae. It is commercially used in films, fibers, medicines and cosmetics for its characteristics which include high viscosity, hydrophilicity and expansiveness. Alginic acid is a linear polysaccharide consisting of 2-1,4-l-guluronic acids and 3-1,4-D-mannuronic acids, the ratio of which varies among species of algae. Compounds which inhibit hyaluronidase [EC 3.2.1.35] activity have often been shown to suppressively affect the degradation of rat peritoneal mast cells as well; for example, certain tannins and pectic substances. In the latter case, the inhibition of hyaluronidase was correlated with the D-galacturonic acid content in the pectic substances. This encouraged the hypothesis that polysaccharides containing uronic acids would be inhibitors of hyaluronidase and/or mast cell degradation. We thus examined various types of alginic acid.

Three types of sodium alginate (types 0.6, 1.0 and 2.0 according to M/G ratio) were obtained, type 1.0 being extracted from Lessoria nigrescens, and type 2.0 from Laminaria angustata. Type 0.6 was a mixture of 40% of the Lessoria nigrescens extract and 60% of the Lessoria frivinics extract, giving an M/G ratio of 3/7. The type 1.0 sodium alginate of molecular weights 388, 372, 314, 259, and 143 kDa were commercially obtained ("Duck Algin," Kibun Food Chemifta Co., Saitama, Japan). Samples with molecular weights of 54, 29, 16, 13, and 11 kDa were obtained by heating the type 1.0 alginic acid at 80-90°C with a jacket heater to give a solid content of 60% for 2, 3, 5, and 7 hours, respectively. Alginate propylene glycol esters of 16 and 54 kDa were commercially obtained ("Duck Loid," Kibun Co.) with low and high values for viscosity, respectively. The molecular weight of each sodium alginate and its derivatives was determined by measuring the limiting viscosity. The inhibitory effect of each algic acid on hyaluronidase was determined by the method described by Sawabe et al., with a slight modification. Sodium chloride (0.1 M) was used as an activator of hyaluronidase instead of compound 48/80 and calcium chloride. The percentage inhibition was calculated as follows:

\[ \text{Inhibition (\%)} = 100 \times \left(1 - \frac{(S - B)}{(C - B)}\right) \]

where \(B\) is the absorbance without an enzyme, \(C\) is the absorbance without an inhibitor, and \(S\) is the absorbance with an inhibitor. The inhibitory activity of mast cell degranulation was measured as the inhibition of histamine release from mast cells. Histamine from the mast cell was released by factor Cytosol of Wistar rats as described by Nakagomi et al. The cells were then incubated at 37°C for 1 h with anti-DNP mouse monoclonal IgE antibodies (Sera Lab. Co., England). After the cells had been washed twice with 5 ml of 0.2% BSA in Tyrode's solution, 10⁶ cells/ml of the IgE-sensitized mast cell suspension was prepared. An antigen (DNP-BSA, 200 ng/ml) was put into wells of a 96-well microtiter plate and incubated for 20 h. The mixture was incubated at 37°C for 5 min, and then for another 10 min with 10 μl of the histamine inducers. After the cells had been centrifuged at 1500 × g for 5 min, the histamine content in the supernatant solution was analyzed by HPLC by a post-column labeling fluorometric reaction with o-phthalaldehyde (Sigma). The percentage inhibition of histamine release from rat peritoneal mast cells was calculated as follows:

\[ \text{Inhibition (\%)} = 100 \times \left(1 - \frac{(To - B1)}{(Co - B1)}\right) \]

where \(To\) is the histamine content of a sample, \(Co\) is the histamine content without a sample, and \(B1\) is the histamine content of a blank.

Hyaluronidase from bovine testis (Sigma, i.e., hyaluronoglucosaminidase, usually exists in an inactive form, and activation by the sodium or calcium ion is needed to induce its maximal activity. Anti-allergic agents such as disodium cromoglycate (DSCG) are known to inhibit hyaluronidase activity. These agents have shown stronger inhibition against the activation of hyaluronidase by metal salts and compound 48/80 than activated hyaluronidase. The inhibitory effects against the activation of inactive hyaluronidase and activated hyaluronidase were measured. As a result, all three types of alginate with a molecular weight of 32 kDa inhibited both the activation of inactive hyaluronidase (Fig. 1A) and activated hyaluronidase (Fig. 1B) to the same degree. This result suggested a difference in the mechanism of action between alginates and anti-allergic agents such as DSCG. Type 1.0 sodium alginates exerted the strongest inhibition in both cases. Next, the effect of the molecular size of type 1.0 sodium alginate was examined. Figure 2 shows that the

---

*To whom correspondence should be addressed (Fax: +81-298-54-6095).

Abbreviations: M/G ratio, molar ratio of D-mannuronic acids to L-guluronic acid; DMBA, 3-dimethylamino-benzaldehyde; DNP, 2,4-dinitrophenyl; BSA, bovine serum albumin; U, β-1,3-glucuronic acids; A, β-1,4-D-acetylglucosamine; ROS, reactive oxygen species.
Alginic Acid Inhibition of Hyaluronidase and Histamine Release

Fig. 1. Inhibitory Effects of Sodium Alginates on the Activity of Hyaluronidase.

44.8 μg/ml of sodium alginate in various d-mannuronate/L-guluronate ratios was added to a hyaluronidase solution before (A) or after (B) adding NaCl as an activator of the enzyme, and the enzyme activities determined. After incubating at 37°C for 20 min the hyaluronidase and alginate, NaCl was added, the solution incubated for a further 20 min, and then hyaluronic acid was added (A). After incubating at 37°C for 20 min hyaluronidase and NaCl, the alginate was added and the solution allowed to stand for 20 min, before hyaluronic acid was added (B). Each vertical bar represents the standard error of the mean (n=5). Duncan's new multiple-range test was performed on each pair of data with SuperANOVA version 1.1 statistics software (Abacus Concepts). The difference between each pair of means was considered significant if p < 0.01.

Fig. 2. Inhibitory Effects of Sodium Alginates of Various Molecular Weights on Hyaluronidase Activity.

22.3 μg/ml of each sodium alginate of differing molecular weight was added to a hyaluronidase solution which had been activated in advance by incubating with 0.17 μM NaCl. The M/G ratio was 1.0. Each vertical bar shows the standard error of the mean (n=5).

higher the molecular weight of sodium alginate, the stronger the inhibition, especially above 150 kDa, although the highest 388 kDa alginate showed weak activity. On the other hand, none of the alginate propylene glycol esters, regardless of their molecular size, inhibited hyaluronidase activity (data not shown). Type 1.0 sodium alginates inhibited rat mast cell degranulation (Fig. 3), although the degree of inhibition was lower than that for hyaluronidase.

Fig. 3. Inhibitory Effects of Sodium Alginates and Their Derivatives on Mast Cell Degranulation.

(A) 22.3 μg/ml of each sodium alginate (M/G ratio = 1) of differing molecular weight was added to the mast cell suspension and an assay as described in the text. (B) 22.3 μg/ml of each alginate propylene glycol ester of differing molecular size was added and an assay conducted. Each vertical bar shows the standard error of the mean (n=5).

As with hyaluronidase, there was a relationship between increasing molecular weight and inhibition. Substitution of the propylene glycol ester for sodium carboxylate in these alginic acids resulted in stronger inhibition.

Hyaluronic acid is a high-molecular-weight polysaccharide composed of alternately-conjugated β-1,4-α-D-N-acetylglucosamine (A) and β-1,3-α-D-glucuronic acids (U), and exists in abundance in the connective tissues of mammals. Hyaluronidase is the enzyme involved in inflammatory reactions, and because anti-allergic compounds sometimes show anti-hyaluronidase activity, substances which inhibit hyaluronidase activity can be expected to have anti-allergic and anti-inflammatory effects. Degranulation of mast cells causes the release of histamine, which is an inducer of immediate hypersensitivity (type I allergy) such as asthma, hay fever and atopic dermatitis. It has not yet been revealed whether or not hyaluronidase participates in the allergic reaction. However, the results obtained with the propylene glycol ester of alginic acids imply that the allergic effects of hyaluronidase are mediated through two different mechanisms in the mast cell pathway, even though these two mechanisms might partially overlap. Pesticic substances and a proteoglycan which had been purified from a mint plant (M. Asada et al., manuscript submitted to J. Agric. Food. Chem.) other than alginic acids had a high content of uronic acids and have been found to be inhibitors of hyaluronidase and mast cell degranulation, so the amount of a uronic acid should be important for its inhibitory activity. It is probable that the inhibition of hyaluronidase by alginic acids is the result of substrate competition, because component sugars of alginic and hyaluronic acids are partially similar, and free or charged carboxyl residues of uronic acids seem to be essential; esterification of the carboxylic acids in alginic acids resulted in no inhibition of the enzyme. To elucidate the exact mechanism involved, a more precise enzymatic analysis is needed.

Although some kinds of polysaccharides are known to have antitumoral activity, type 1.0 sodium alginate did not inhibit the growth of either a murine myeloleukemic cell line (P388D1) or spontaneously transformed human umbilical cord vein endothelial cell line (ECV 304) (data not shown).

References