Journal of Inclusion Phenomena **5** (1987), 525–534.

Binding Properties of Alginic Acid and Chitin

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(Received: 15 October 1986; in final form: 28 February 1987)

Abstract. The binding properties of granular alginic acid(H-alg.) and chitin to iodine, bromine, cadmium ion, calcium ion and cholesterol were investigated. Chitin-iodine and chitin-bromine compounds closely resemble those of H-alg. The amount of iodine included by these polysaccharides increased with a decrease in the concentration of potassium iodide (KI). The number of sugar residues bound to one iodine molecule extrapolated to 0 g of KI was around 6.0 for H-alg. and 6.4 for chitin. These saccharides, which do not form a gel in water, were found to also absorb KI and radioactive iodide or iodine in aqueous solution. H-alg. did not show as much affinity to cadmium ion and calcium ion and cholesterol in iso-propyl alcohol as metal-alginates.

Key words: Alginic acid, chitin, iodine, bromine.

1. Introduction

During the course of an investigation of the binding properties of sodium alginate to metal ions and metal alginates to iodine, cholesterol and dyes [1, 2], it became desirable to have experimental values for several measurements of granular alginic acid (H-alg.) obtained from the treatment with hydrochloric acid. Sodium alginate is a binary heterogeneous copolymer of D-mannuronate (M) and L-guluronate (G) residues arranged in a blockwise pattern along the linear chain. There are three types of blocks, M, G and MG blocks in which these two uronic acids occur in some sort of alternation [3]. The ability of alginates to form gels in the presence of calcium ions depends mainly upon auto-cooperatively formed junctions which were interpreted in terms of an "egg-box" model between chain regions enriched in G-sequences [4]. The size of beads or films of metal alginate becomes shorter due to the shrinkage of the gel upon chelation.

By treating sodium alginate with hydrochloric acid, the polysaccharide acquires the form of alginic acid with the appearance of helicoidal structures which are associated supplementarily [5]. X-ray diffraction analysis [6, 7] gave the fibre repeating distance for sodium alginate, poly G-acid and poly M-acid as 15 Å, 8.7 Å and 10.35 Å, respectively. Such differences, reflecting packing differences, are typical of polysaccharide chains. Among crystalline A-, B- and V-amyloses which have a packing behavior based on the helical molecular structure [8], only V-amylose forms an iodine-inclusion compound because of its rather short helix pitch, that is, its rather wide cavity [9].

The metal alginate fibre is very sensitive to acid. Electron microscopic evidence [10] indicated that under acid condition even on treatment with 2% osmic acid solu-

Presented at the Fourth International Symposium on Inclusion Phenomena and the Third International Symposium on Cyclodextrins, Lancaster, U.K., 20–25 July 1986.

tion at the time fixation, the shrinkage of the fibre occurred. The formation of a metal alginate iodine compound at about pH 1, the removal of cholesterol from acetic acid by the addition of metal alginate and the packing of some blocks of dye powder enclosed in a metal alginate net without dissolving in alcohol after acid addition which were reported in the previous paper [1] could be explained by the shrinkage of the metal alginate fibre under the acidic condition.

On the other hand, chitin obtained from the carapace is a popular adsorbent [11] or a starting material for the production of adsorbing media like the amino-acid substituted glucans [12], and is insoluble in water and does not form a gel in water. Being different from the structure of alginic acid, chitin is poly-β-(1,4)-*N*-acetyl-D-glucosamine. Three polymorphic forms have been identified by X-ray methods. Among α-, β- and γ-chitin, α-chitin appears to be the most stable form and consists of a series of sheets of twofold chains [13].

The present investigation was undertaken to study the binding properties of H-alg. and chitin to iodine, bromine, cadmium ion, calcium ion and cholesterol.

2. Materials and Methods

2.1. MATERIALS

Sodium alginate ... Sodium alginate was obtained from Wako Pure Chemical Industries, Ltd. G/M of this sample is ca. 0.91 and $\overline{DP}$ is 468–473.

Granular alginic acid(H-alg.) ... H-alg. was prepared from the treatment with hydrochloric acid of sodium alginate by the same procedure described in the previous paper [1].

The acidity of the solutions in which 50 mg and 500 mg of H-alg. prepared using 1N HCl was immersed in 50 ml of water were pH 3.3 and pH 2.7, respectively, but for fresh H-alg. the pH was 4.2–4.5. The granular sample was washed with water until the rinsing no longer gave a chloride ion reaction in the preparation, although it showed some evidence of the presence of Cl^-.

Chitin ... Chitin was obtained from Wako Pure Chemical Industries, Ltd. This sample obtained from carapace contains 5–7% of nitrogen and shows less than 10% loss on drying over sulphuric acid. This sample was used without further purification.

The acidity of the solutions in which 50 mg and 500 mg of chitin was immersed in 50 ml of water were pH 4.1 and pH 3.5, respectively, but for the sample washed with water the solution showed pH 6. Purification of chitin requires treatment with dilute acid to remove carbonate, although this technical grade chitin was negative to the Cl^- reaction.

Cd or Ca-alginate beads ... In a typical experiment, 25 ml of 0.4% sodium alginate was added dropwise to 50 ml of 0.01M cadmium nitrate or calcium nitrate with stirring. After standing for 24 h at 30°, alginate beads were separated by a nylon cloth.

Cholesterol detectable solution ... In the previous experiment the cholesterol content of the solution was determined by the ferric chloride reaction. The method lacks reproducibility. The most versatile method of cholesterol detection is with an enzyme. In this paper a one step enzymatic method called the COD · DAOS color

former method was used. (Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of H_2O_2, which oxidatively couple with 4-aminoantipyrine and PhOH in the presence of peroxidase to yield a chromogen with max. absorption at 600 nm.) This cholesterol test kit named the Cholesterol E-Test was obtained from Wako Pure Chemical Industries, Ltd.

All other commercially available chemicals used were of the highest available purity.

2.2. METHOD

Iodine ... The weighed amount (0.3–3.0 g) of sample in a glass-stoppered conical flask was mixed with 18 ml of a 0.15M iodine-potassium iodide solution (KI 5, 10 and 15 g/100 ml) and 2 ml of 1N HCl. In one part of the iodine experiment, 30 ml of 0.15M I_2 solution was used. After storing for 24 h, an aliquot of the supernatant liquid was taken out with a pipette and the amount of iodine consumed by the sample was determined by absorbance at 285 nm or by titration with 0.1N $Na_2S_2O_3$.

For the experiment with radioactive iodine, three kinds of radioactive solutions were prepared by spiking with radioactive sodium iodide-^{125}I solution (aqua, KI (5 g/100 ml) and 0.15M I_2). The specific radioactivity of the radioiodine added was so high that its weight was negligible. The activities of the solutions were adjusted to about 2×10^4 cpm/ml, each 15 ml of radioactive solution was mixed with the sample and after standing for 1 h, the activity of the supernatant liquid was measured with a Aloka Auto Well ARC-500.

After removal of the solvent, the solid phase was dried in air until a constant weight was recorded. For X-ray analysis the solid phase sample ground into a powder or a standard material (I_2, KI, H-alg. and chitin) powder was spread on a Cellophane tape and the tape was attached to the sample window. The mesurement and the scanning electron microscopy (SEM) investigation were also carried following the same procedure described in the previous paper [1] respectively.

Bromine ... The method used here was virtually identical with that described for iodine, but the latter was replaced with 30 ml of 0.029M Br_2 (chitin) or the mixture of 27 ml of 0.05M Br_2 and 3 ml of 1N HCl (H-alg.). After storing for 5 h (chitin) or 6 days (H-alg.) in the dark, the amount of bromine consumed by the sample was examined both by absorbance at 394 nm and titration with 0.1N $Na_2S_2O_3$. Ca-alg. beads prepared with 30 ml of 0.02M $Ca(NO_3)_2$ and 20 ml of 0.4% Na-alg. were immersed into the mixture of 20 ml of 0.025M Br_2 and 2 ml of 1N HCl. Several parallel experiments using different concentrations of Br_2 in the solution were carried out.

Metal ion ... To investigate the pH effect on the binding ratio, 25 ml of 0.2% Na-alg. or the mixture of 25 ml of water and 50 mg of H-alg. was added dropwise with stirring to the mixture of 25 ml of 0.01M $Cd(NO_3)_2$ and 25 ml of buffer solution (pH 4 and pH 7 ... standard buffer solution for a pH meter obtained from Wako Pure Chemical Industries, Ltd. pH 8.3 ... 0.1M NH_4Cl 16:0.1N NH_4OH 1. pH 9 ... 0.1M NH_4Cl 4:0.1N NH_4OH 1. pH 10 ... 0.1M NH_4Cl 1:0.1N NH_4OH 4. pH 11 ... 0.1M NH_4Cl 1:0.1N NH_4OH 32) in a beaker. The pH of the mixture was

measured and the electrode of the pH meter spiked in the solution was washed with 10 ml of water. The mixture was left to stand for 20 h at 40° and filtered. After rinsing the precipitate, the filtrate and the wash liquid were mixed and the amount of metal ion in the solution was determined by titration with 0.01M EDTA. To investigate the metal concentration effect on the binding ratio, metal alginate-beads were immersed in 50 ml of 0.01–0.14M $Cd(NO_3)_2$ or $Ca(NO_3)_2$ for 24 h, and then the solution was adjusted to pH 1 with 1N HCl. After standing for 24 h, a 10 ml portion of the supernatant liquid was taken out and the amount of metal ion consumed by the sample was determined by titration.

Cholesterol ... Each weighed sample (0.05–0.5 g chitin and H-alg.) was mixed with 25 ml of cholesterol standard solution (150 mg/isopropyl alcohol 1000 ml). After shaking for 10 min and standing for 1 h, a 1 ml portion of the supernatant liquid was taken out and transferred into 3.0 ml of the cholesterol E-Test solution. The mixture was shaken vigorously and left to stand for 5 min at 37°, and then the extinction was measured at 600 nm. For every run of absorbance, titrating and radioactivity experiments a blank solution was made and measured simultaneously.

3. Results and Discussion

Iodine ... Both Chitin and H-alg. react with iodine to give dark purple stained adducts. In this paper results for chitin are presented. Figure 1 shows the relation between the amount of chitin added and the concentration of iodine. The numerical value of the absorbance or the titration decreased with an increase in the amount of chitin. The amount of chitin corresponding to the iodine used was calculated

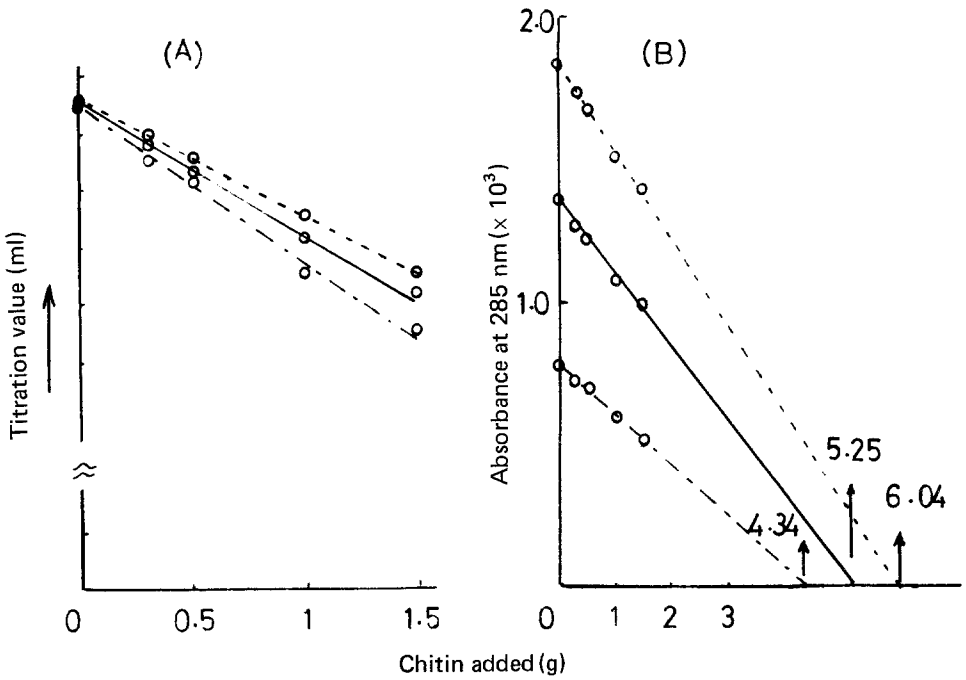

Fig. 1. Effect of the amount of chitin added on the concentration of iodine (A) by titration with $Na_2S_2O_3$ and (B) by UV absorbance. KI content in I_2 solution —·—0.05, ——0.10 and ··· 0.15 (g/ml).

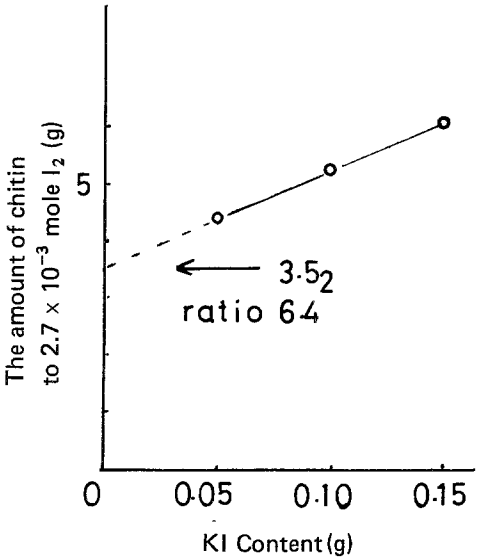

Fig. 2. The amount of chitin bound to iodine.

from the slope obtained from the least squares fit of the plots. The value increased with an increase in the content of potassium iodide as shown in Figure 1(B). In Figure 2 the values obtained were plotted against the KI content, and the number extrapolated to zero g of KI was also indicated. It shows that 6.4 residues of chitin correspond to one iodine molecule in the chitin-iodine compound. Taking into account the impurity of the chitin sample the value is in good agreement with the value (6) reported before for the H-alg.-iodine compound.

The proposed model for the helicoidal structure of alginic acid by Simionescu *et al.* [5] showed the same binding ratio for the H-alg.-iodine complex as ours. On the other hand, the adsorption of iodine and bromine on chitosan, but not on chitin has been reported [14]. The binding ratio for chitosan-halogen complexes showed 0.5 molar halogen per one hexosaminyl residue in the proposed structure, in which a halogen molecule links to two amino groups of chitosan to form a bridged ladder like structure. The structure differs from the well-known amylose-iodine complex in which iodine atoms are aligned in channels of the polysaccharide.

Table I. Weight of chitin before and after experiment

Chitin taken (g)	0.500	1.000	3.000
Chitin after experiment (g)	0.680	1.310	3.759
Iodine content of the chitin $-I_2$ adduct (g)	0.084	0.149	0.350

In Figure 3 X-ray powder diffractograms are shown. The presence of potassium iodide was shown by the peak at 25°. The characteristic peak observed in the chitin-iodine sample corresponds to 3.16 Å. This value differs from the value of 3.06 Å observed for the I-I distance of iodine packed in the helical cylinder of the

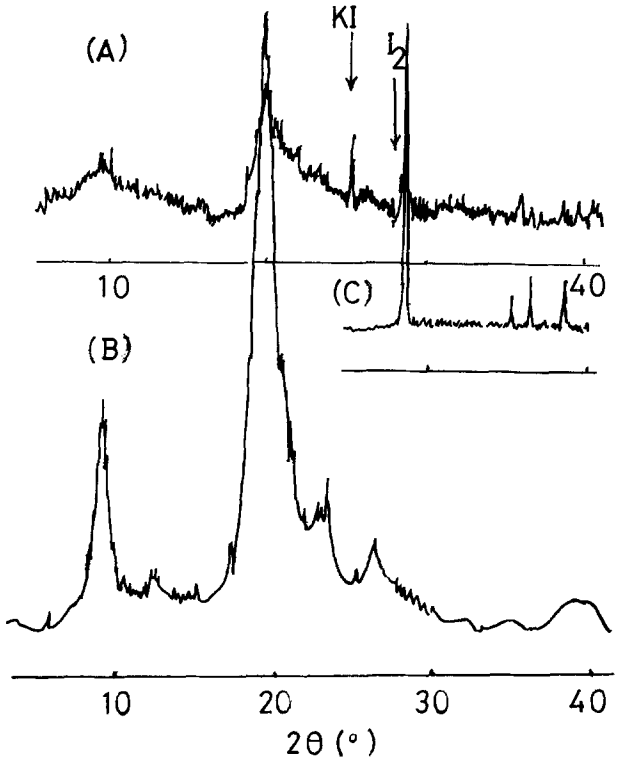

Fig. 3. X-ray diffractograms of (A) chitin-iodine (B) chitin and (C) iodine.

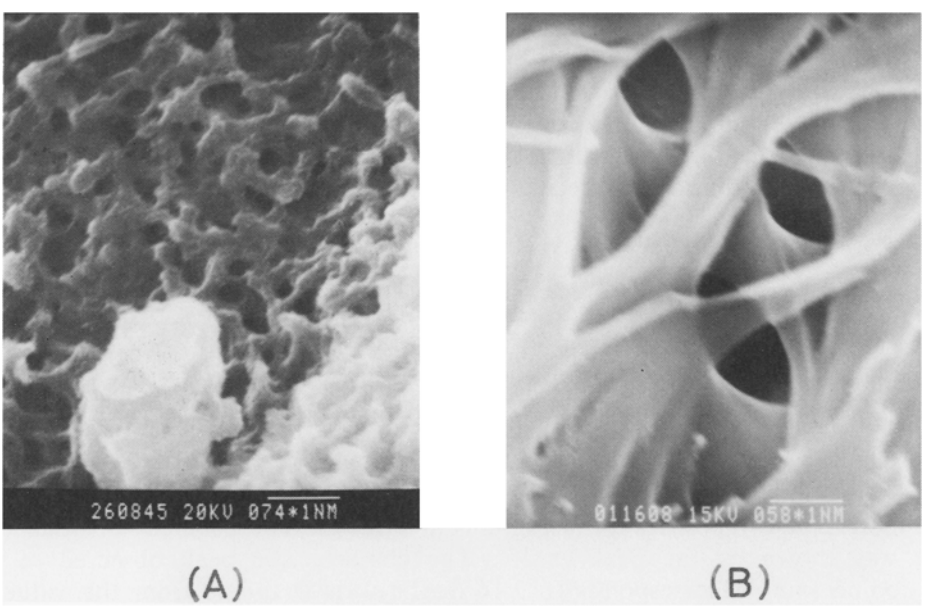

Fig. 4. Scanning electron micrographs of iodine-poly saccharides (A) H-alg., (B) chitin.

α-cyclodextrin-iodine complex [15] slightly but resembles those in the Ca-alg.-iodine compound (3.13 Å) and the Cd-alg.-iodine compound (3.17 Å) prepared at pH 1 [1]. The results by X-ray diffraction [16] for the chitin sample used here agree with an orthorhombic unit cell of α-chitin with dimensions $a = 4.76$ Å, $b = 10.28$ Å, and $c = 18.85$ Å. This fibre repeating distance of about 10.3 Å is the same as that for poly M-acid. H-alg. does not possess as high a stereoregularity as chitin, although the fibre repeat distance of 8.7 Å showed clearly [16]. Therefore, it is very likely that the fiber axis of poly G-acid contains two D-guluronic acid residues per turn, which is the repeat obtained when two α-(1,4)-linked residues in the stable 1 C conformation are arranged in a 2_1 helix [17].

Figure 4 shows the porous structure of iodine-poly saccharide. The microscopic structure of chitin is very similar to that of granular alginic acid.

The results of the experiments for radioactive iodine are shown in Figure 5. In a radioisotopic exchange reaction, the activities are divided in portion to weight among exchangeable species at equilibrium, so the activities reduced by these saccharides are affected by various factors. As shown in Figure 5, the reduction of the plotted line is quite reasonably attributable to the reversible equilibrium between the solid phase and the liquid phase. Chitin and H-alg. also absorb radioactive iodide and radioactive iodine. Chitin absorbs them faster than H-alg.

Bromine. In this experiment with bromine the absorption equilibrium between the solid phase and the liquid phase was attained after 1 hr for chitin but after 5 days for H-alg. Although the limited solubility of bromine in water precludes measurements at high concentrations, as shown in Figure 6, these saccharides absorb bromine (A) and bead form Ca-alg. has a high binding ratio corresponding to the concentration of bromine (B). Being different from chitin, chitosan absorbs bromine in water up to 1.12 molar bromine per two hexosaminyl residues [14] and neither bromine nor iodine is adsorbed on *N*-acetylchitosan [14]. The latter conflicts with our results for the chitin-iodine and chitin-bromide adducts.

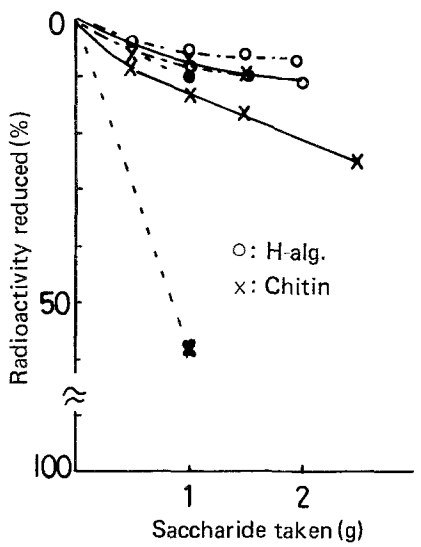

Fig. 5. Effect of poly saccharides on ^{125}I-solution ··· aqua, ——I_2 and —·—KI.

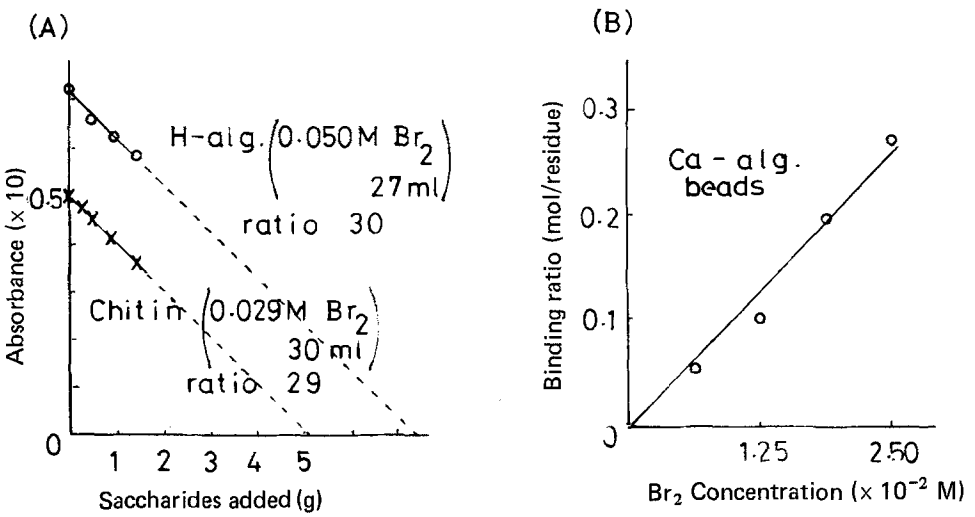

Fig. 6. Effect of sample form on the binding ratio of (A) H-alg. or chitin to Br_2 and (B) Br_2 to bead form Ca-alg.

Metal. In Figure 7(A) results for Na-alg. and H-alg. are presented. Chitin scarcely reacted with 0.1M and 1.0M $Cd(NO_3)_2$, respectively. The binding ratios are similar in the range pH 3–8 and then increase markedly up to pH 10. Taking into account the amount of sample used (H-alg. 2.8×10^{-3} mole, Na-alg. 2.5×10^{-3} mole) the two values are the same. According to Rees [18] alginates gel reversibly and are therefore suspected of an ability to form an ordered conformation when stabilized by cohesion with other chains. They are formed by the controlled mixing of the polysaccharide with the salt of a suitable cation and liquified by ion exchange with an alkali-metal cation such as Na^+ or by sequestering the gelling cation. The ring conformations are known to be as shown for both the solid state and solution. The chains are ribbon-like and extended, and they pack like planks in a timber-yard. The chains have twofold screw symmetry. This conformation for poly-G [19], appears to persist [20] in all of the salt forms so far studied. Mackie *et al.* [21], reported that the conformation of polyguluronate remains unchanged in the condensed and solution phases.

Figure 7(B) shows the different reactivities of metal alginates, H-alg. and Na-alg. with Cd^{2+} or Ca^{2+}. The high affinity of metal alginate to metal ion may be caused by dispersion in the gel net. These bindings by the "egg box" model conformation and the gel net of metal alginates suggest nothing about the formation of a dark blue iodine-H-alg. adduct or a dark purple iodine-chitin one. With regard to the binding sites of Cu^{2+} in chitin, examination of the crystal structure of α-chitin suggests that a site consisting of two N and two O ligands is more probable [22]. The size of a chitin particle effects the adsorption of dyes [23] which was explained as intra-particle diffusion [24]. These reports indicate that the binding mechanism of chitin to iodine is different from those.

Figure 7(A) shows no evidence of the binding of H-alg. to Cd^{2+} at pH values below 3. pH 2.85 is the acidity at which a sample of poly-G acid is prepared from the treatment with hydrochloric acid by Haug's method [25]. X-ray diffraction

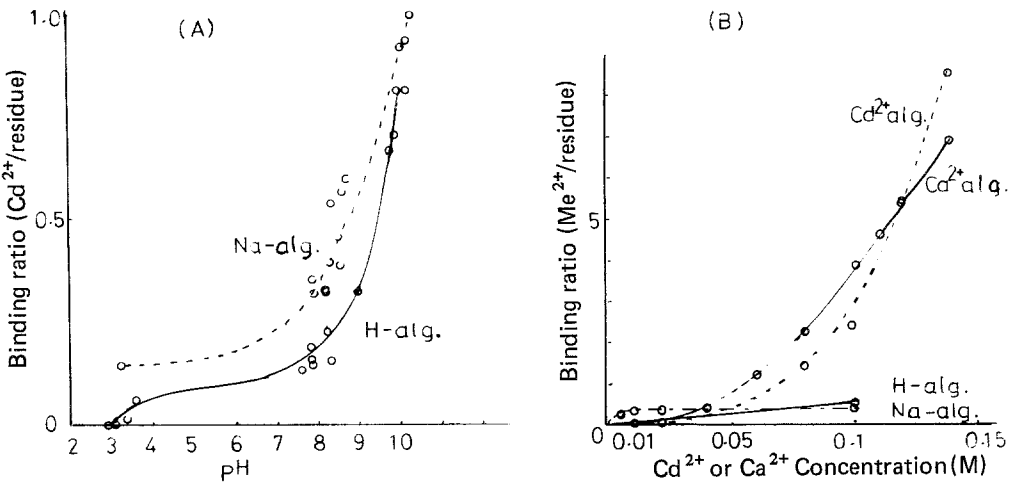

Fig. 7. Changes of binding ratio (A) pH-induced (B) M^{2+} concentration-induced.

points to the high degree of crystallinity of alginic acid, obtained after gel dehydration (achieved by hydrochloric acid addition) as against the altogether amorphous sodium alginate [5]. On the other hand α-chitin may be obtained by treating β-chitin with 6N HCl [17]. No method is known to reverse this change. These data suggest that the formation of metal alginate-iodine observed at pH 1, H-alg.-iodine or chitin-iodine compounds depends on the fiber chain conformation of these polysaccharides at the acidity below pH 2.85.

Cholesterol... None of chitin was obtained pure, so the sample of chitin obtained from carapace was positive to the cholesterol E-Test and the extinction to standard cholesterol solution increased with an increase in the amount of chitin, but any reduction of cholesterol by chitin was not observed at all. In the previous experiment the removal of cholesterol from acetic acid solution by the addition of metal alginate beads was observed. Such reduction of cholesterol from iso-propyl alcohol by the addition of metal alginate beads (unpublished), H-alg. or chitin is not observed in this experiment, so it indicates that the removal of cholesterol from the acidic solution depends on the shrinkage of the metal alginate beads, but not on the fiber chain packing.

These results suggest that the binding capacity of H-alg. or chitin is fixed, so the amount of iodine enclosed in H-alg. or chitin decreases with an increase in the amount of KI.

4. Conclusions

It may be concluded from the results of this investigation that crystalline alginic acid and chitin have almost the same binding ability to iodine, bromine, cadmium and cholesterol. Chitin-iodine and chitin-bromine compounds closely resemble those of crystalline alginic acid. The amount of iodine included by these polysaccharides decreases with an increase in the amount of potassium iodide. These polysaccharides which do not form a gel in water also absorb potassium iodide and radioactive iodide or radioactive iodine, but do not have an affinity to cholesterol. H-alg. does not

have as high a binding ratio to cadmium ion and calcium ion corresponding to the concentration of the nitrate metal ions as metal alginate shows after the treatment with hydrochloric acid. The affinity of H-alg. to 0.01M Cd^{2+} is zero around pH 3.

Acknowledgements

The author wishes to thank Prof. N. Ikeda and Dr R. Seki of the University of Tsukuba for carrying out this radioactive iodine experiment.

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